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VOLUMEN XIII

UITGEVERIJ Dr. W. JUNK — DEN HAAG — 1960

VOLUMEN XIII

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Fasc. 2. (pag. 81—160) edit: 30—VII—1960
Fasc. 3. (pag. 161—264) edit: 31—X—1960
Fasc. 4. (pag. 265—376) edit: 15—XII—1960
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Printed in Holland by
Dijkstra's Drukkerij n.v., voorheen Boekdrukkerij Gebroeders Hoitsema, Groningen

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PERITHECIUM-FORMATION OF MICROSPORON GYPSEUM AND ITS COGNATE, EPIDERMOPHYTON RADIOSULCATUM VAR. FLAVUM SZATHMÁRY 1940 ON SOIL.

by

Dr. SEBASTIAN SZATHMÁRY ¹) & Dr. ZSOMBOR HERPAY ²)

Communication of the Mycologic Institute of the University of Medical Sciences, Debrecen and of the Mycologic Laboratory, Karcag Hungary (Leader: Dr. Sebastian SZATHMÁRY)

PRELIMINARY COMMUNICATION.

(29.II.1960)

M. gypseum has been demonstrated from soils by means of VANBREUSEGHEM'S keratinophylic method by many research workers. However, the related publications confined themselves to the definition of this species and have not dealt with those variants which can often be observed in soil-cultures.

The perfect, perithecium-forming tribes have not been handled so far, which are especially important from botanical standpoint.

Our method essentially consisted of the use of woman-hair and of different animal-hairs sterilized by dry heat /248—266° F./ and conserved in 75 % strong methyl or ethyl-alcohol, laid upon the soil to be examined. We very often obtained this fungus too, as well as its variants, and other species of fungi, from soils of very different sorts.

For a year, since we have been performing soil-cultures we could observe that both the *M. gypseum* and its variants, though they are to be found anywhere, are permanent and, in addition, abundantly occurring inhabitants of certain types of soils.

They are mostly plentiful in soils disturbed in their biological equilibrium or in fields cultivated and manured, as well as in arable land. Among these they are profusely contained in plough-lands of maize, corn or in flower-beds fertilized with cowdung, but they are scarcely to be found in soils of biological equilibrium, i.e. in the upper strata of the soil which have not been disturbed, so in mea-

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¹) Candidate for Medicine.

²) First Assistent

dows, lucerne-(alfalfa) fields and sometimes in forest-soil as well. As a general rule these upper strata of soils rich in decaying organic matters contain them more abundantly than the lower ones, in which the members of the species-group *Ctenomyces* and its cognates are more plentiful present than the former ones.

The existence of the Aleurospores in different kinds of soils may claim interest, since we can draw important conclusions from them as well as from the joint occurrence of the perfect and variant formations of the same Dermatophyte-species, which are sometimes associated with other fungi.

In this respect we found remarkable that the very large frequency of the members of the *M. gypseum* contrasted sharply with the infections of humans group caused by them, quite in a similar way as it was reported in 1940 by SZATHMÁRY in connection with the *T. fluviale* and recently by AJELLO concerning the *Keratinomyces ajelloi*.

This extremely different manifestation of the pathogenicity, concerning generally only one single variant formation, proves, that the prototypes of the *M. gypseum* and its cognate species-groups, to which also the *Keratinomyces ajelloi* and the *E. radiosulcatum var. flavum* (SZATHMÁRY) belong, are living in the soil as saprophytes. They may become parasites only under specific conditions.

In view of the circumstance that infections on human skin by this species of fungi were occurring almost without exception by way of the soil of gardens or chicken yards, it may be supposed that the development of the pathogenicity must take place in specific soiledaphons.

In addition, the parallel existence of the typical tribes and their variants in the same soil may be regarded as a trend to variations, which is known as common feature of the Dermatophytes.

It seems also to be of importance, that among the typical tribes there occur some which are known to be more apt to form perithecia than their next relative, imperfect forms. The comparison of the pecularities of the mycelial system of all these variants show an unbroken continuity of transition among one another and those variants, whose most accomplished shape is the perithecium. The simultaneous presence of these transitory forms can be observed also in the same colony. In the mycelium of the imperfect forms there are filaments which are forming the perithecial framework or basket and producing those special fine threads which appear as decorations on the basket and endow them with the botanical features characteristic for the species.

In the present communication we are going to deal mainly with the structure, framework and ornamentation of the perithecium of the *M. gypseum* (*Ach. gypseum*), which seems to be of importance both from the point of view of the botanical classification of this fungus as well as its next cognate, the *E. radiosulcatum var.*

flavum and from the distinction of these two fungi from each other.

The different types of perithecia, which we came to know in connection with the soil-cultures, led us to the assumption that the perithecium formation of the fungi living in the soil is the only valid criterion, which may be adopted as a basis for any botanical and scientific classification. Those knots of spores which may likely shape details of perithecia, but do not show at all the perithecial ornamentation and framework, are of no consequence. This is why we strove to give the definition of pertithecium-types which had developed on the surface of the soil they are regarded typical and in botanical respect characteristic for the family of fungi.

Among the Dermatophyte-perithecia, hitherto discovered by Szathmáry, the first one is the perithecium of the *Ctenomyces trichophyticus* (i.e. the prototype of the *T. gypseum*), which is an organ of fructification distinguished by its chamois-horn-shaped formations (fig. 1), spirals (fig. 2), finger-biscuit forms (fig. 3), and with its curved threads woven into one complex network as it were a perithecium-basket (fig. 4). The special ornaments of this species can determine also in themselves the place in the botanical classification of this fungus between *Ctenomyces* and *Myxotrichum.*

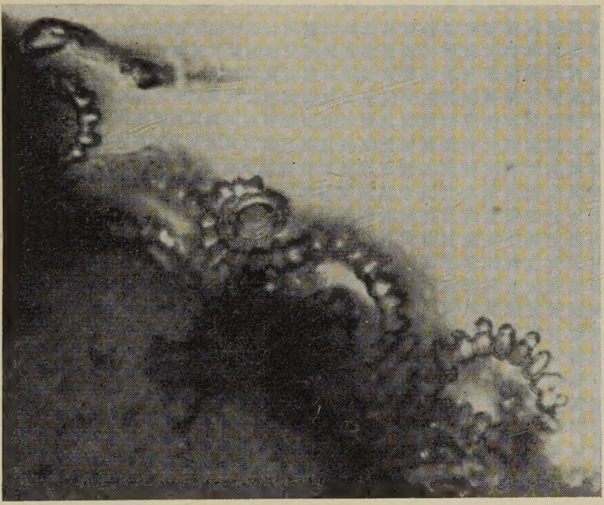


Fig. 1

Quite different is the perithecium-framework and ornamentation of that class of fungi, which belong to the *Myxotricha*. The feature of their ornaments is a kind of tapering ends of filaments winding in tortuous lines with hooks on their curved ending (figures 5-6).

The framework of the perithecium of the *Achorion quinckeanum* is composed of filaments divergent on their thickened endings,

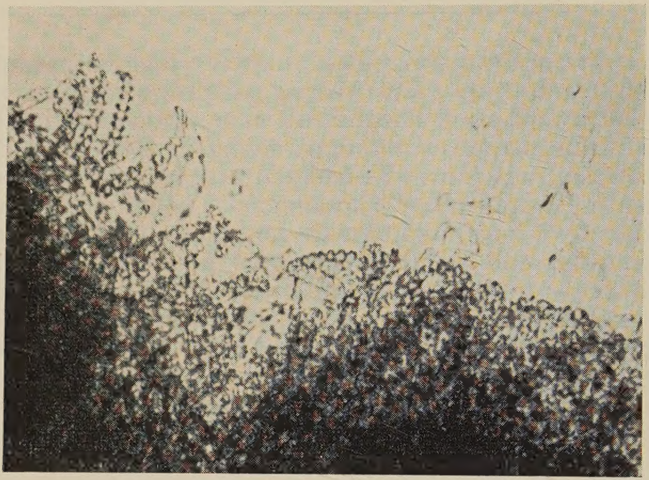


Fig. 2

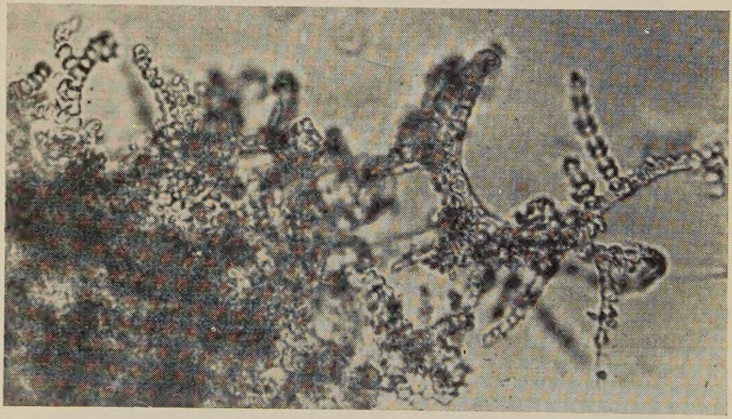
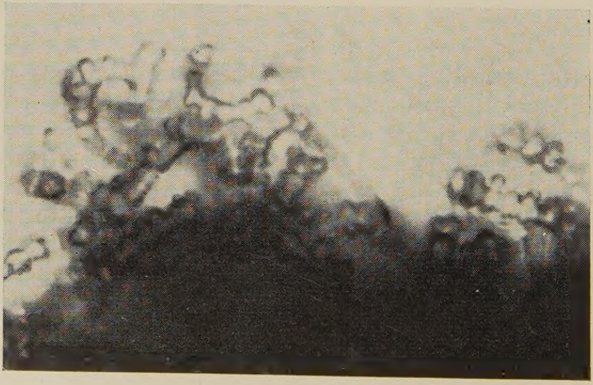


Fig. 3



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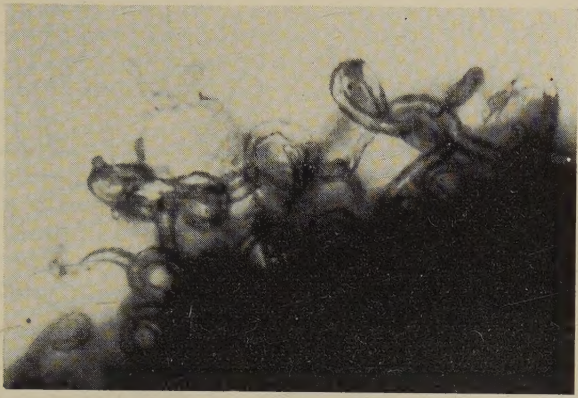


Fig. 5

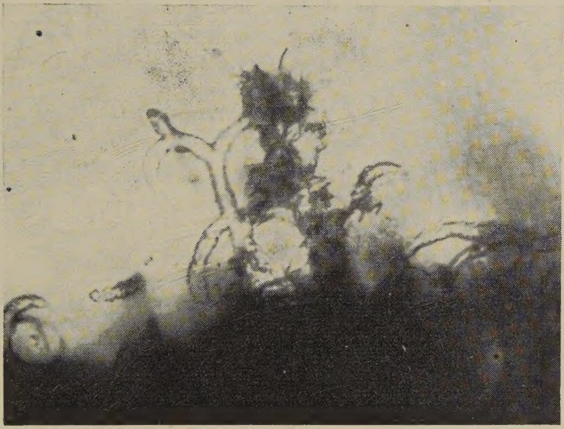
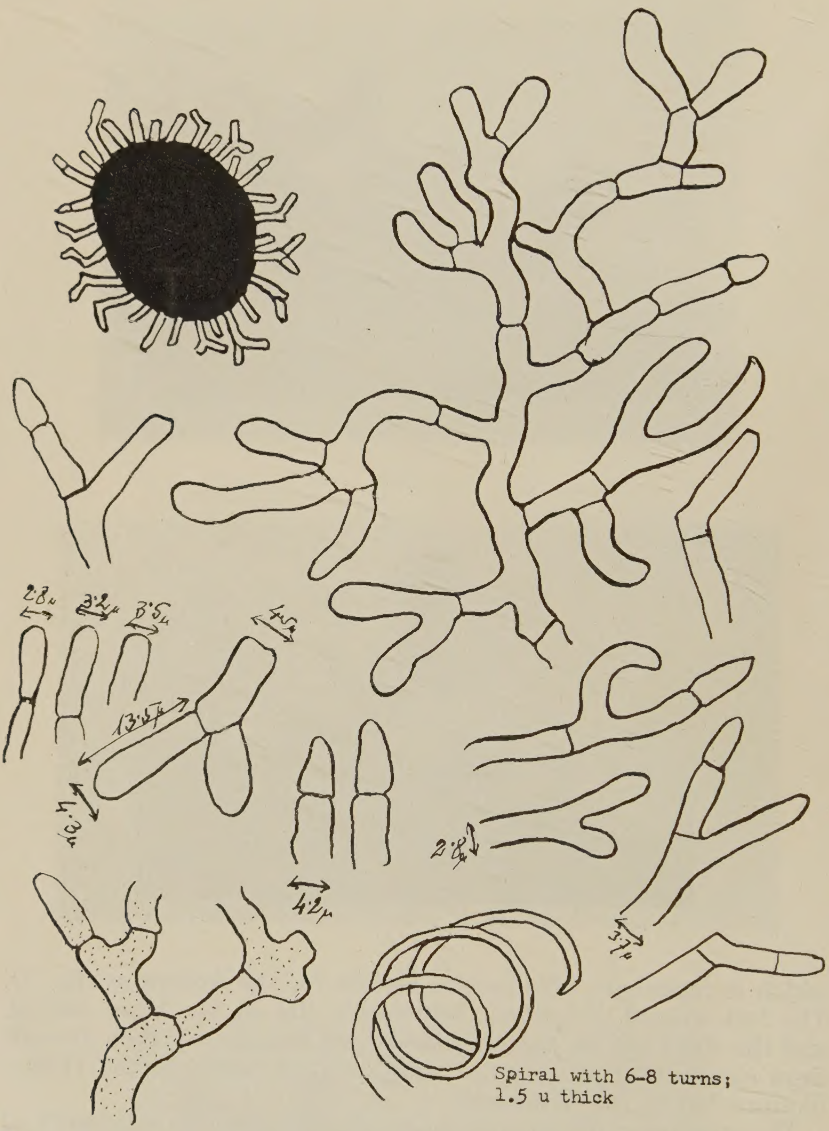


Fig. 6

which is regarded as distinctive feature for the Achorions (fig. 7). The fork-shaped dichotomic bifurcation (fig. 8), the blunt ending, and the short obtuse bayonet-like lateral branches of their threads show clearly that this class of fungi does not belong to the Trichophytons but to the Achorions.

The structure and ornamentation of the perithecium-network of the *M. gypseum* and its next-related cognate, the *E. radiosulcatum var. flavum* is essentially different from those described above and indicates rather more a presumable relationship to the Trichophytons than to the Achorions. The framework of the perithecia of these species of fungi is, on the whole, identical, therefore this relationship is regarded as a fact beyond doubt.

The *M. gypseum* and the *E. radiosulcatum var. flavum* are considered as next related fungi both on account of their macro- and micro-



Spiral with 6-8 turns;
1.5 u thick

Fig. 7—8. Perithecium of *Achorion quinckeanum* and its thick structural mycelia.

culture, since there is only the difference between them, that the latter fungus species has got plenty of spirals deriving mostly of a common stem (tendril), whereas the former ones dispose of none of them.

Apart from the difference in their macrocultural features which can suffice alone to separate these two species of fungi, there appears also a considerable difference in the spiral structure of the micro-cultures in the ornamentation of their perithecia. In this respect the distinctive ornament with the perithecium of *E. radiosulcatum var. flavum* is the spiral which cannot be found on the perithecium of the *M. gypseum.*

Peculiar to the imperfect colonies of the soil-cultures of the *M. gypseum* is a gray, loosely woven, almost cobweblike mycelium, on which are sitting the rusty brown coloured, dumpy, and densely granulated closterospores, containing 4 or 5 loculi. These spores are often located at the end of lateral sporophores originating from the same main branches (fig. 9). The thallus is composed on the whole from filaments of the same thickness. A feature of its hyphae is the frequent racquet-formation. The perithecium-formation of the perfect forms manifests itself at first in the more frequent occurrence of the thick filaments and in the condensation of the mycelial system composed by them. The articulation of filaments becomes a constant feature. Beside the little fawn-coloured balls produced by the condensation of filaments, which are getting darker and darker by aging, there occur also such concentrations which are created without any mycelial system of the imperfect forms. They have the appearance of independent and separated knots without any globular shape and a size with a diameter of

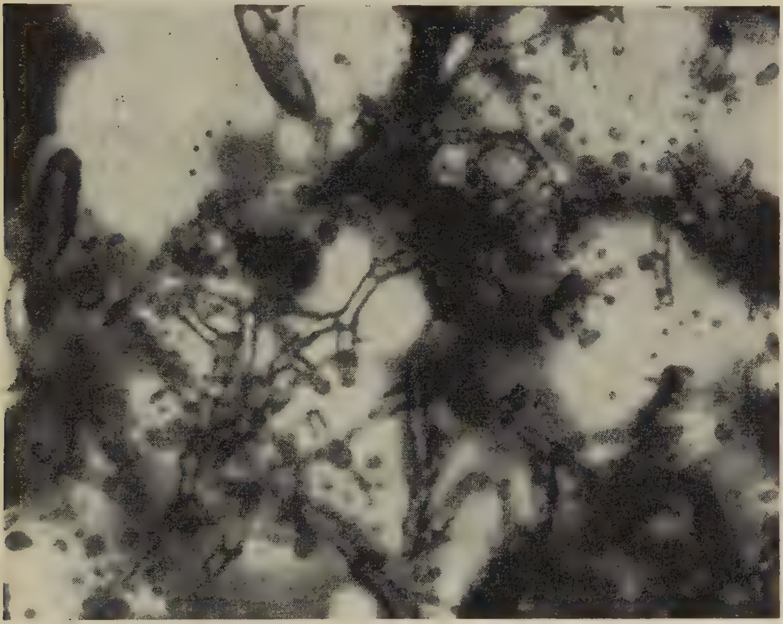


Fig. 9.

0.2—1.5 mm. These are formations without any filaments and mostly of dark rost colour. On their surface there usually are to be seen through microscope also the rost-coloured closterospores typical of these fungi (fig. 10). Between the extremes of the perithecium-formation, mentioned above, there are in most cases also the transitory forms to be found. The rostcoloured spherical perithecia are the most perfectly developed ones. They seem to be detached and grown independently from the loose mycelial system. They are all the more advanced forms, because they are not empty frameworks but abundantly contain asci, while the former for the most part are empty. The structure of the perfectly developed perithecia consists of thick, septate,mostly curved filaments, connected with one another as ring-like threads which may entirely prevent the asci from dispersion being kept by them together (fig. 11). The terminal parts of the thick mycelia serving as decoration of the perithecium and making its framework are not curved as a rule, or at least less curved as the threads participating in the shaping of the basket. The former ones are ending in a blunt or dichotomic bifurcation. (fig. 12). Some of the threads making up the framework have the shape of a finger-biscuit on account of a mild contraction in their middle part. (fig. 13.). Their outside, thickest part is 6—7 μ broad, their length makes 18—20 μ. Their surface is sparsely granulated. It seems, that the granulation may develope in old filaments.

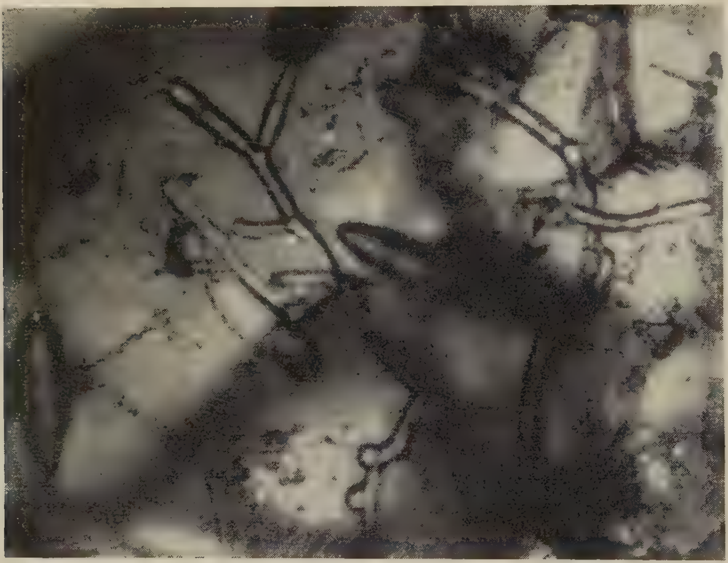


Fig. 10.

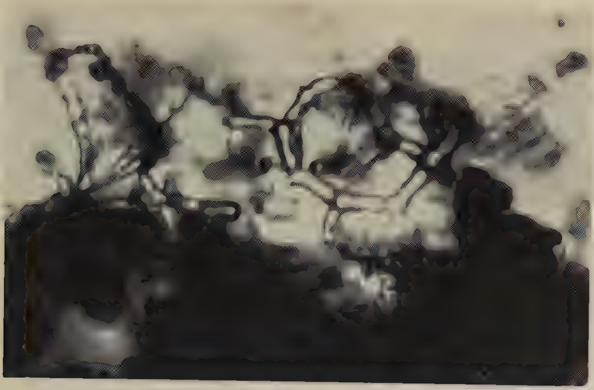


Fig. 11

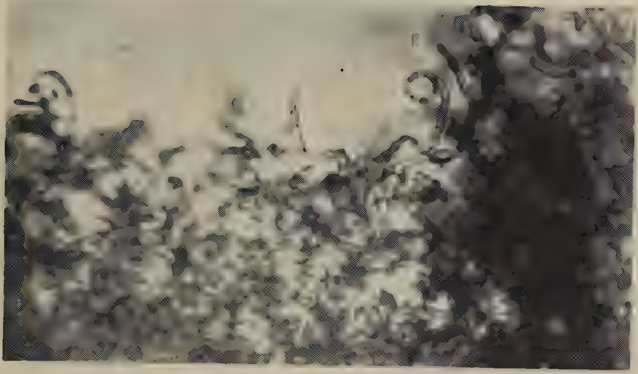


Fig. 12

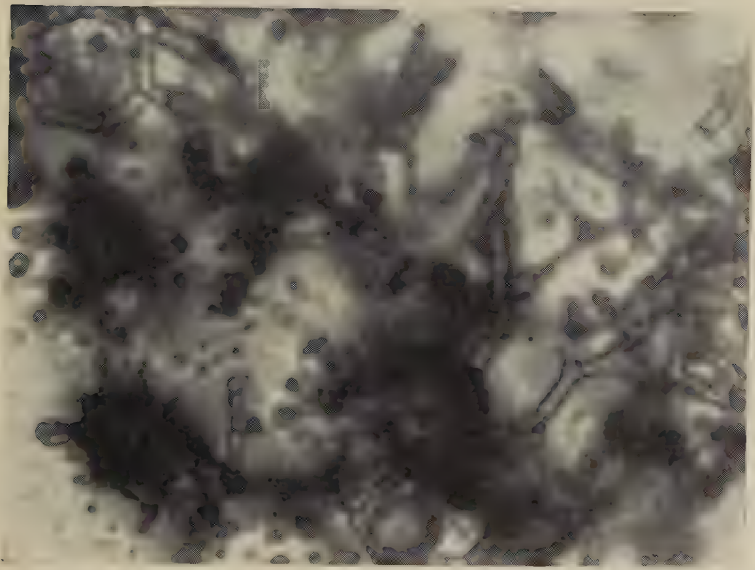


Fig. 13.

The asci are round, with thin cell walls, having a diameter of 6 μ on the average and containing 6 spores. Their ascophores are mostly short and ovate, sometimes globular with a smooth surface, internally without any structure, having a size of 3 × 2 μ, resp. 3 × 3μ on the average.

That these perithecia belong to the species *M. gypseum* is demonstrated not only by the fact that this fungus has been developed from the ascophores on artificial carbohydrate agar culture-medium, but we managed in some cases to induce the *M. gypseum*, coming directly from human skin lesion, to perithecium formation on soils sterilized with heat. The perithecia produced this way proved to be identical with those originated spontaneously as well as in cultures on laboratory nutrient substrate.

The development of the perithecia of the *E. radiosulcatum var. flavum* is quite the same as that of the *M. gypseum*. Their structure is similarly identical. (figures 14 and 15). Their granula and smooth mycelia forming the peridium have also the shape of finger-biscuits (fig. 16). with a breadth of 6—7 μ on their widest part and a length 16—20 μ,

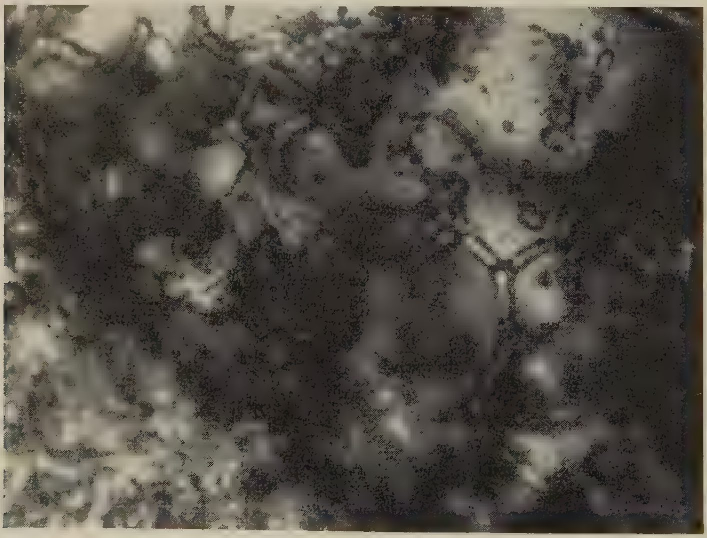


Fig. 14

The thin filaments without granulation are 4 μ broad, while their spirals are 1.6—2 μ thick. The latter are often like tendrils (figures 15, 16 and 17), but there exist also those with many convolution. Their asci are blackberry-shaped balls with 6 μ diameter on the average, containing 6 spores. These are short, ovate or globular with a smooth surface. Their size averages 3 × 2 μ resp. 3 × 3 μ (fig. 17).

The closterospores existing on the surface of the perithecium are

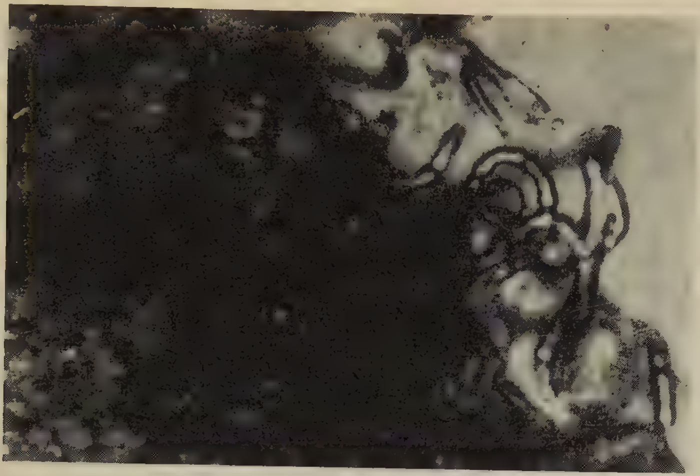


Fig. 15

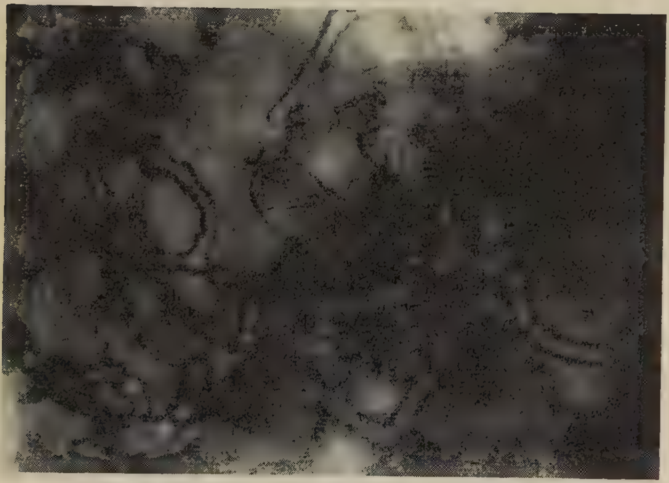


Fig. 16

macrospores of an average size of 10 × 40 μ, mostly with 6 cells and with densely granular surface, ending bluntly. Their microspores are pear-shaped and of an average size of 3 × 5 μ, resp. 2 × 5 μ, more rarely.

Perithecial structures often can be found both in *M. gypseum* and in *E. radiosulcatum var. flavum* without asci. One can see also perithecium-formations with more primitive forms, or even some consisting only of closterospore agglomerations. Between these simple forms and the completely developed perithecia there are also a number of transitional forms to be observed.

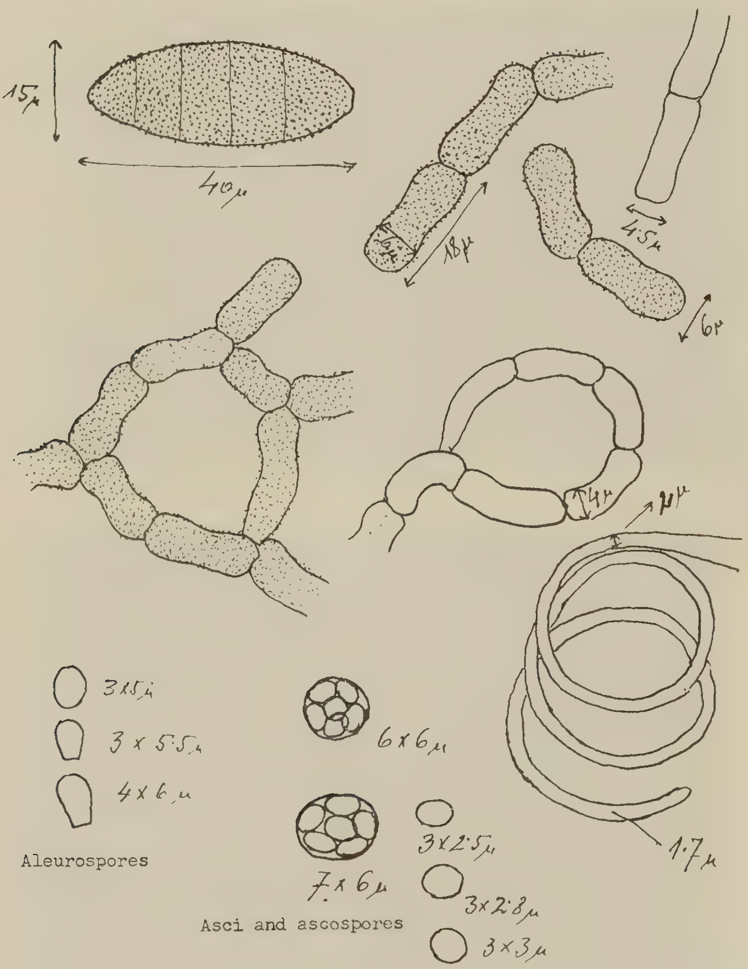


Fig. 17. Details of the perithecium of *Epidermophyton radiosulcatum* var. *flavum* SZATHMÁRY, 1940.

The joint presence of both the imperfect and perfect forms of these two species of fungi is very frequent in the same soil not only with each other but also with other species groups of Dermatophytes. *M. gypseum* can generally be found together with *E. radiosulcatum var. flavum* along with perithecia and imperfect forms of the *Ctenomyces serratus* (fig. 18), *Ctenomyces trichophyticus* aleurosporic groups of fungi living in the soil and with Gymnoascaceae as well. (fig. 19).

The knowledge of the joint presence of the species of fungi known as pathogens can elucidate the origin of polymycotic infections.

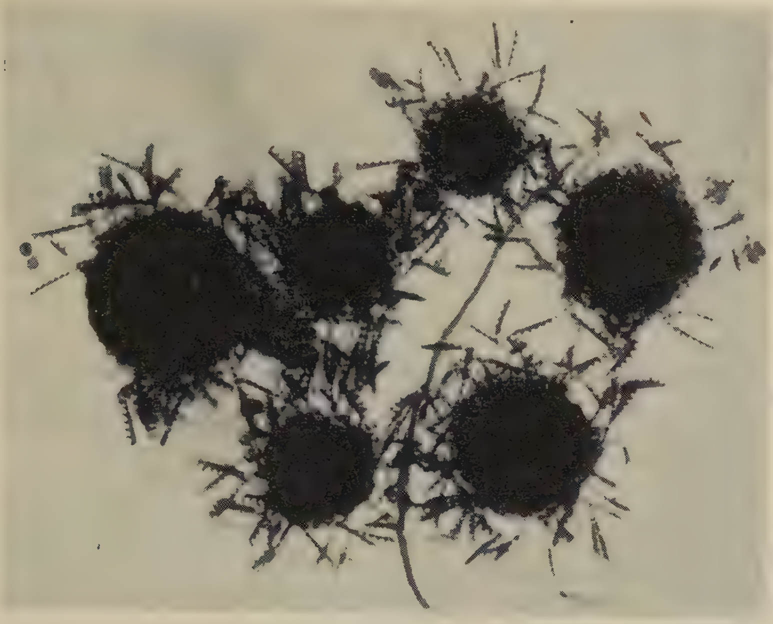


Fig. 18

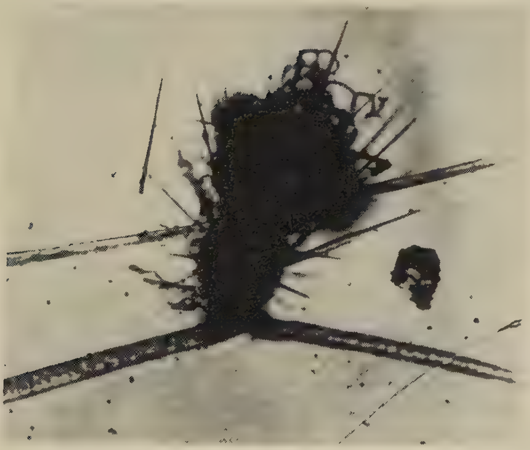


Fig. 19

SUMMARY

The successful elicitation of the perfect form of fructification in *M. gypseum* and in its cognate, *Epidermophyton radiosulcatum var. fla-*

vum SZATHMÁRY, 1940 from the soil is reported on. The method consisted of using human and/or animal hairs laid upon the soil to be examined. The hairs were sterilized in dry heat and conserved in 75% methyl-or ethyl alcohol.

The present communication mainly deals with the structure, framework and ornamentation of the perithecium of *M. gypseum* and *Ep. radiosulcatum var. flavum.*

The joint presence of both the imperfect and perfect forms of these two species is very frequent in the same soil as well as their common occurence with other species-groups of Dermatophytes.

ZUSAMMENFASSUNG

Es wird über die erfolgreiche Züchtung des perfekten Fruchtkörpers bei *M. gypseum* und, bei dem ihm nahe verwandten *Ep. radiosulcatum var. flavum* SZATHMÁRY, 1940 mittels Erdbodenkulturen berichtet.

Die Methode benutzte menschliche und tierische Haare, die einfach auf den Erdboden gelegt worden sind. Die Haare waren erst durch trockene Hitze sterilisiert und dann in 75% Methyl- oder Äthyl-alkohol aufbewahrt.

Die jetzige Mitteilung behandelt hauptsächlich die Struktur, das Gerüst und Verzierung des Fruchtkörpers in den zwei benannten Gattungen.

Das gleichzeitige Vorkommen der imperfekten und perfekten Formen der zwei Gattungen im Erdboden ist sehr häufig, ebenso wie ihre Vergesellschaftung mit anderen Gattungen der Dermatophyten.

SUR L'ORIGINE TELLURIQUE DU CTENOMYCES INTERDIGITALIS (EPIDERMOPHYTON KAUFMANN-WOLF). DYSIDROSE PALMAIRE PAR CONTACT AVEC DE LA TERRE

par

R. Evolceanu, I. Alteras, A. Dobrescu & V. Kursky-Eremia

(*Bucarest*)

(12.I.1960)

Assez récemment on a découvert que, parmi les moisissures saprophytes du sol, il y en a qui sont, en même temps, des parasites comme, par exemple, le *Histoplasma capsulatum* et l'*Allescheria boydii*, agents de certaines mycoses profondes (1). Mais les champignons pathogènes, qui paraissent y être les plus répandus, comme saprophytes, sont les dermatophytes, notamment le *Microsporum gypseum* et le *Keratinomyces ajelloi* (1, 5, 6). La présence, dans le sol, de dermatophytes, autres que les deux espèces sus-citées, a été mentionée seulement d'une façon isolée .Depuis peu,Evolceanu & Alteras y ont isolé une espèce d'*Aleurisma* (7). Cette espèce, qui paraît être largement répandue dans le sol, inoculée à l'homme, a provoqué un herpès circiné typique. Mais ce n'est pas un dermatophyte commun, puisqu'il n'a été isolé des mycoses spontannées que d'une façon exceptionnelle. Parmi les autres dermatophytes isolés du sol, mention est faite du *Ctenomyces mentagrophytes*, qui avait été isolé d'une cave par Lurie & Borok (12) en 1955. En 1940, l'auteur hongrois Szathmáry a isolé, de la vase de certaines rivières, un dermatophyte qui, d'après ses caractères morphologiques, devrait appartenir au genre *Ctenomyces*. Ce dermatophyte, très voisin botaniquement au *Ctenomyces mentagrophytes*, a été désigné, par le sus-dit auteur, sous le nom de *Trichophyton fluviale* (8, 14). D'après lui, ce serait probablement la forme primitive du *Ctenomyces interdigitalis*. Toutefois, comme il va être démontré plus loin, le *Trichophyton fluviale* ne peut être identifié complètement au *Ctenomyces interdigitalis*. Il en diffère par son affinité pour le follicule pileux et par certains caractères de la morphologie macro- et microscopique de ses cultures (voir "Discussions").
Contrairement au *Trichophyton fluviale*, le *Ctenomyces interdigitalis* n'envahit pas le follicule pileux. Il a une grande affinité pour la

plante des pieds et, par la suite, c'est justement lui qui devrait être plus spécialement cherché dans le sol. En effet, nul territoire cutané n'est exposé, d'une façon si continuelle, au contact avec la terre, que celui des pieds. Les chaussures ne peuvent pas empêcher ce contact, car elles sont facilement traversées par la poussière de terre et, de plus, créent les conditions locales d'humidité favorables au développement des champignons. Bien que, d'après les considérations ci-dessus, la large présence du *Ctenomyces interdigitalis* dans les couches superficielles du sol soit très probable, elle n'a pas été mentionnée encore, dans la littérature.

Observations personnelles

L'année passée, au mois d'avril, une femme, âgée de 34 ans, vient consulter l'un de nous, à l'hôpital dermato-vénérologique de Craiova, pour une dyshidrose de la main gauche. L'éruption, qui n'était pas bien délimitée, était constituée de petites vésicules ayant chacune les dimensions d'un grain de millet. Elle occupait presque la moitié de la face palmaire de la main, ainsi que le 3-ème et le 4-ème espace interdigital, ne dépassant guère les premières falanges des doigts. La dysidrose datait depuis l'hiver dernier. La malade déclara, qu'afin de nettoyer la bouche d'arrosage située près de la maison, elle creusa de sa main gauche le mélange de glace, de terre et de feuilles mortes qui s'y trouvait. Une dizaine de jours plus tard elle observa l'apparition de l'éruption palmaire mentionnée plus haut. L'examen microscopique précisa la nature parasitaire de l'affection. A l'occasion d'un voyage à Bucarest, la malade nous fut amenée pour un examen mycologique plus complet et nous trouvâmes dans les vésicules palmaires de nombreux filaments mycéliens.

Les cultures, que nous avons obtenues par l'ensemencement sur le milieu d'épreuve de Sabouraud des vésicules palmaires, étaient, pendant les premiers jours, blanches et duveteuses et se développaient assez rapidement. Petit à petit elles devenaient jaunâtres et poudreuses, moins poudreuses toutefois que les cultures du *Ctenomyces mentagrophytes*. Examinées au microscope, les microcultures en goutte pendante nous montrèrent de nombreuses aleuries sphériques ou pyriformes, disposées en acladium ou en grappe, des fuseaux à plusieurs compartiments et à l'extrémité légèrement arrondie, ainsi que des vrilles serrées. En somme, les traits morphologiques macro- et microscopiques de ce champignon étaient ceux de *Ctenomyces interdigitales* (*Epidermophyton interdigitale* = *Epidermophyton* de Kaufmann-Wolf).

Essais d'isolation du Ctenomyces interdigitalis des couches superficielles du sol

Une certaine quantité de terre, contenant des feuilles mortes et de petites branches pourries, a été prélevée de l'endroit indiqué par la malade comme source de la contamination. Cette quantité de terre nous l'avons distribué en 20 boites de Petri, en mettant au-dessus d'elles des fragments d'ongles, préalablement stérilisées par ébullition[1]), et de cheveux. Les cheveux et les ongles étant envahis par d'autres champignons kératinophyles que le *Ctenomyces*, champignons parmi lesquels le *Microsporum gypseum* occupa la première place, nous fûmes obligés de renouveler notre essai. En procédant une seconde fois à cette même expérience, nous observâmes, après 3 semaines environ, le développement d'un petit duvet blanc sur quelques cheveux et sur quelques fragments d'ongles. Examiné au microscope, ce duvet nous montra la présence de quelques aleuries qui rappelaient, par leur aspect et par leurs dimensions, les aleuries propres au genre *Ctenomyces* (fig. 1). En ensemençant ce duvet sur le milieu d'épreuve de Sabouraud, nous avons réussi, après plusieurs passages et essais d'isolation successifs, à obtenir une culture duveteuse, blanche, semblable, par ses caractères macro- et microscopiques, aux cultures du *Ctenomyces interdigitalis*.

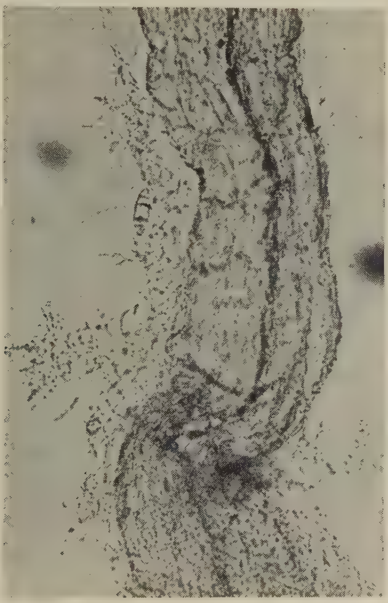


Fig. 1. Poil envahi par le *Ctenomyces interdigitalis* après contact avec de la terre. (On y aperçoit aussi une macroconidie appartenant à l'ubiquitaire *Microsporum gypseum*).

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[1]) Le procédé utilisé par nous a été imaginé par VANBREUSEGHEM (17). Cet auteur, en partant de la kératinophylie des dermatophytes, réussit à isoler du sol ces champignons, par la mise en contact d'une certaine quantité de terre avec des organes riches en kératine, tels que cheveux, ongles, plumes etc. Ces organes étant invadés seulement par des champignons kératinophyles, parmi lesquels les dermatophytes, l'isolation de ces derniers devient facile.

Caractères morphologiques du dermatophyte isolé du sol

Sur le milieu d'épreuve de Sabouraud, les cultures de notre champignon étaient blanches et duveteuses (fig. 2). Elles s'y développaient assez rapidement. Après 7—8 jours, leurs diamètre mesurait déjà plus de 2 cm. Deux ou trois semaines plus tard, les cultures devenaient légèrement poudreuses et jaunâtres, comme les cultures du parasite isolé des vésicules palmaires de notre malade. Les cultures en goutte pendante (chambre humide de Van Tieghem), examinées au microscope, montraient, elles aussi, les caractères morphologiques des cultures du champignon isolé de la malade, ça veut dire les mêmes aleuries globuleuses ou pyriformes (fig. 3), les mêmes fuseaux à plusieurs compartiments, plutôt obuts à leur extrémité libre (fig. 4). Dans les vieilles cultures on pouvait observer des vrilles serrées (fig. 5) ainsi que des chlamydospores terminales.

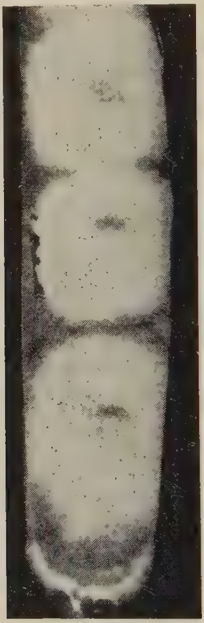

Fig. 2. *Ctenomyces interdigitalis* isolé du sol. Culture de 10 jours sur milieu glucosé de Sabouraud.

Inoculations experimentales du dermatophyte isolé du sol

Les inoculations expérimentales furent effectuées à la souris blanche, au cobaye et à l'homme. Dans ce but, on a utilisé une culture de 8—10 jours, sur milieu d'épreuve de Sabouraud. Les essais

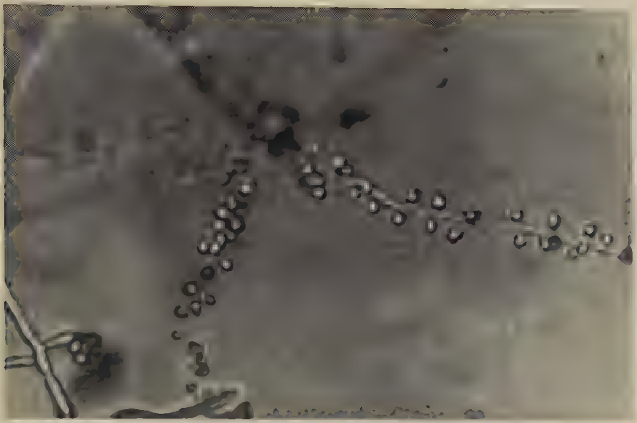

Fig. 3. Aleuries en acladium de la souche isolée du sol. (Microculture de 15 jours).

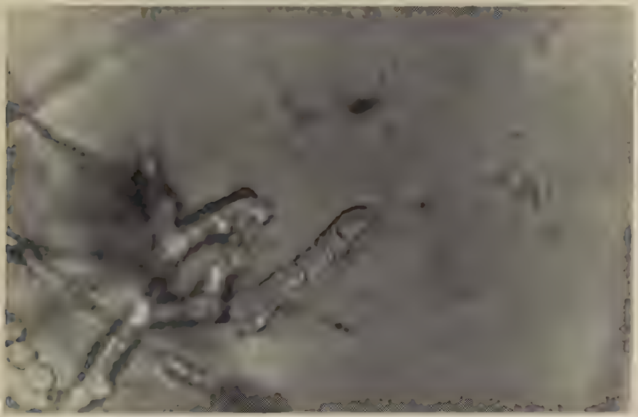

Fig. 4. Fuseau à extrémité arrondie (la même culture que dans la figure précédente).

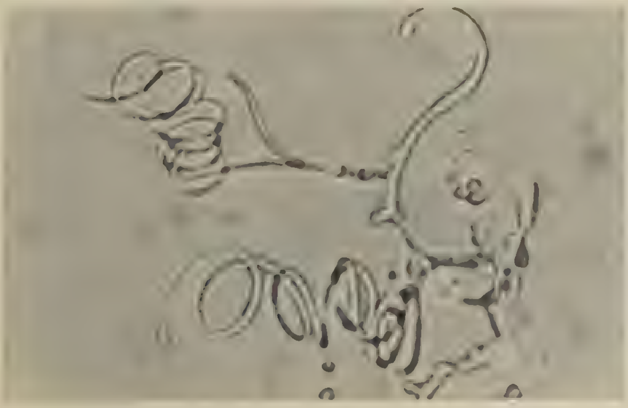

Fig. 5. Vrilles serrées. Microculture de 5 semaines.

d'inoculation à la souris blanche (5 individus) n'ont point réussi. Des 5 cobayes inoculés à la peau du dos, l'un a présenté, au bout de 8 jours, une petite tache érythémateuse, partiellement couverte de squames dines et de petites croûtes. Les autres sont restés indemnes. Les squames contenaient de nombreux filaments mycéliens, mais la plupart des poils étaient respectés et on n'en voyait que de très rares parasités. Le type parasitaire était le type ectothrix, des filaments mycéliens et des chaînes d'arthrospores pouvant être observés seulement à la surface de ces poils.

Les inoculations effectuées à l'homme (à la face antérieure de l'avant-bras) ont été suivies par l'apparition, au bout de 9 jours, d'une plaque érythémato-vesiculeuse, dont les vésicules contenaient de nombreux filaments mycéliens cloisonnés et ramifiés.

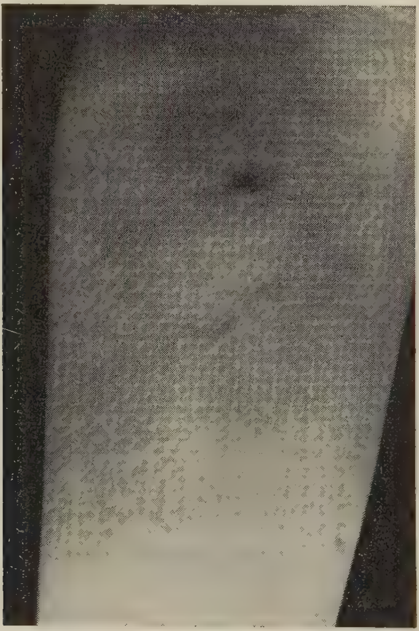

Fig. 6. Inoculation expérimentale à l'homme de la souche isolée du sol. On y aperçoit aussi une lésion folliculaire banale.

Les jours suivants la plaque est devenue plus grande, de nouvelles vésicules se sont formées dans la partie périphérique (fig. 6), tandis que la partie centrale s'affaissait et commençait à guérir. Une pomade fongicide, à l'acide salicylique et benzoique, amena la guérison de la lésion expérimentale, au bout d'une semaine.

Les rétrocultures, obtenues par l'ensemencement des squames et des vésicules, récoltées des lésions experimentales du cobaye, ainsi que des lésions expérimentales de l'homme, ont reproduit exactement la souche inoculée.

Propriétés immuno-biologiques de la souche isolée du sol

Des cultures de la souche isolée du sol on a préparé un extrait spécifique[1]) (une ''trichophytine''). L'intradermo-réaction, pratiquée avec cette trichophytine, a été fortement positive chez 5 personnes atteintes de kérion. Elle a été moins caractéristique, plutôt douteuse, chez 3 personnes atteintes de dysidrose parasitaire, palmo-plantaire.

Discussions

Comme il a été démontré plus haut, on a réussi a prouver chez une jeune femme l'origine tellurique d'une dysidrose parasitaire, palmaire, due au *Ctenomyces interdigitalis*. En effet, ce dermatophyte a été isolé, non seulement des lésions de la malade. Il était présent, en même temps, dans le sol, à savoir, à la place indiquée par la même malade, comme source probable de la contamination. La souche, que nous avons isolée de la terre, montrait tous les caractères mycologiques et pathogéniques du *Ctenomyces interdigitalis*: la même morphologie botanique, macro- et microscopique, ainsi que le même aspect des lésions provoquées par son inoculation expérimentale (et accidentelle) à l'homme et à l'animal. Macroscopiquement, les cultures ne ressemblaient pas complètement ni aux cultures du *Ctenomyces mentagrophytes*, qui sont plus plâtreuses, ni aux cultures de l'*Achorion* (*Trichophyton*) *quinckeanum*, plus blanches et plus duveteuses. La souche que nous avons isolée de la terre, nous n'avons pas réussi de l'inoculer à la souris et, par conséquent, ne peut pas être considérée comme appartenant à l'*Achorion quinckeanum*. Il en résulte que cette dernière espèce peut être éliminée de notre discussion, bien que, d'après VANBREUSEGHEM, sa présence dans les couches superficielles du sol soit très probable (18). Les caractères microscopiques de notre souche n'étaient, non plus, parfaitement semblables à ceux de *Ctenomyces mentagrophytes*, car les aleuries y étaient plutôt pyriformes que sphériques.

Bien entendu, les caractères morphologiques, macro- et microscopiques des cultures, n'auraient pas parmis, elles seules, de différencier rigoureusement le *Ctenomyces interdigitalis* du *Ctenomyces mentagrophytes*, car ces deux espèces sont très rapprochées et même considérées parfois identiques du point de vue botanique. Restent les caractères pathogéniques. Cas caractères nous ont permis, peut-être mieux que les caractères morphologiques, de considérer notre champignon comme appartenant au *Ctenomyces interdigitalis* et non au *Ctenomyces mentagrophytes*. Les vésicules palmaires n'étaient pas, chez notre malade, disposées en cercle, autrement dit, l'éruption n'avait pas l'aspect d'un herpès circiné typique, comme cela aurait été le cas s'il s'agissait du *Ctenomyces mentagrophytes*. Par contre, les vésicules étaient disposées plutôt au hasard. La lésion expérimentale humaine était non folliculaire et, chez le cobaye inoculé, il n'y avaient que très peu de poils malades. Ces poils étaient parasités suivant le type strictement ectothrix. Les propriétés pathogéniques mentionnées sont celles du *Ctenomyces interdigitalis* et pas celles de *Ctenomyces mentagrophytes*.

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[1]) Habituellement, nous préparons la trichophytine d'après une méthode utilisée en bactériologie pour obtenir les toxines microbiennes. La masse mycélienne des cultures vieilles de 5 à 6 semaines, est lavée, puis deshydratée par le maintien en acétone 24 heures, séchée et finement triturée. La poudre qui en résulte, est délayée dans de l' eau distilée, en proportion de 1 à 9, maintenue à 80°C, pendant une heure, et centrifugée. Le surnatant est soumis à la dialyse à l'eau courante, pendant 2 jours, à puis l'eau distilée et, enfin, séché.

Le fait d'avoir démontré la présence, dans les couches superficielles du sol, du *Ctenomyces interdigitalis*, vient à l'appui de l'opinion émise dès 1922 par ALEXANDER, - cité par SZATHMÁRY (14) opinion d'après laquelle le *Ctenomyces interdigitalis* serait un simple saprophyte du sol. Comme nous l'avons déjà vu, le *"Trichophyton fluviale"*, isolé par SZATHMÁRY, n'a été considéré, ni par cet auteur, identique au *Ctenomyces interdigitalis*. Les cultures en étaient poudreuses et l'inoculation à l'homme et à l'animal avait provoqué des lésions folliculaires. Il paraît donc que le *Trichophyton fluviale* soit très voisin, sinon identique au *Ctenomyces mentagrophytes*.

D'autre part, il faut tenir compte du fait que, en vue d'isoler le champignon de la vase des rivières, l'auteur hongrois avait mis cette vase directement en contact avec la peau, préalablement traumatisée, du cobaye. L'apparition, chez le cobaye, de la mycose, a été interprétée comme une preuve certaine de l'existence du *Trichophyton fluviale* dans la sus-dite vase. Mais la possibilité d'une mycose due à un *Ctenomyces mentagrophytes* déjà présent sur la peau du cobaye, et rendu pathogène par le traumatisme de l'inoculation, ne peut être éliminée d'une façon absolue. Nombre d'auteurs, parmi lesquels MACKINNON (13), FUENTES & ABOULAFIA (9), EVOLCEANU & AVRAM (4), KOCH & RIETH (11) ont trouvé que,chez le cobaye, les mycoses dues au *Ctenomyces mentagrophytes* sont assez fréquentes et revêtent le plus souvent une allure épizootique. Toutefois, il paraît beaucoup plus probable que le *Trichophyton fluviale* de SZATMÁRY fut présent dans le sol et qu'il ne préexistait sur la peau du cobaye, car la mycose s'y manifesta trop régulièrement après l'inoculation de la vase et, de plus, l'aspect des cultures du *Trichophyton fluviale* n'était pas absolument superposable à celui des cultures du *Ctenomyces mentagrophytes*. D'ailleurs, le mérite de l'auteur hongrois est aussi celui d'avoir été parmi les premiers qui ont isolé les dermatophytes du sol et d'avoir insisté sur leur présence dans ce milieu.

Vue la probabilité de la large présence et même de l'ubiquité du *Ctenomyces interdigitalis* dans les couches superficielles du sol, cette espèce devrait être recherchée avec beaucoup de persévérence. In vitro elle paraît être moins kératinophyle que d'autre moisissures du sol (le *Microsporum gypseum* par exemple), fait dont on doit tenir compte. Si l'ubiquité de ce champignon dans le sol venait s'être démontrée, alors sa nature primitive ne ferait plus aucun doute.

La propriété du *Ctenomyces interdigitalis* de parasiter seulement la peau et de respecter les poils, paraît confirmer cette primitivité, car, d'après ce que l'on sait, les dermatophytes attaquent d'abord la peau et, seulement en second lieu, les poils. L'hypothèse contraire, défendue par certains auteurs, notamment par SIGALOVA (15), hypothèse d'après laquelle le *Ctenomyces interdigitalis* serait un *Ctenomyces mentagrophytes* qui aurait perdu la capacité de parasiter les poils, nous semble moins vraisemblable. Tout de même, cette dernière hypothèse ne peut être complètement éliminée, elle non plus. Le *Microsporum gypseum*, qui est extrêmement répandu dans le sol et, par conséquent essentiellement primitif, peut parasiter la peau tout aussi bien que les cheveux et les poils. Mais la disposition de ce parasite dans les cheveux est très variable. Il n'y a pas un type parasitaire bien défini, comparable à celui qu'on observe chez les espèces communes du genre *Microsporum*. Cela prouve que le *Microsporum gypseum*, lui aussi est encore loin d'être parfaitement adapté à parasiter les cheveux.

Le débat reste ouvert. Comme nous l'avons déjà dit, afin de résoudre le problème concernant la primitivité et la nature saprophytique du *Ctenomyces interdigitalis*, il faudra continuer de le rechercher, avec persévérence, dans les souches superficielles du sol.

RÉSUMÉ

Les auteurs ont réussi à isoler, de la terre, le *Ctenomyces interdigitalis* (*Epidermophyton interdigitale*, *Epidermophyton* de KAUFMANN-WOLF). Les caractères morphologiques, macro- et microscopiques des cultures, ainsi que ceux des lésions expérimentales, obtenues par son inoculation à l'homme et à l'animal, ont précisé qu'il s'agissait bien du *Ctenomyces interdigitalis* et non du *Ctenomyces mentagrophytes* ou du *Trichophyton* (*Achorion*) *quinckeanum*. La souche isolée avait provoqué une dysidrose palmaire chez une femme âgée de 34 ans. La malade s'est contaminée en creusant, de sa main gauche, la terre mélangée à des feuilles mortes qui se trouvait dans une bouche d'arrosage. D'après la littérature consultée, il semble que ce dermatophyte n'a pas été isolé, directement, jusqu'à présent, des couches superficielles du sol, bien que sa présence dans ce milieu fut admise par nombre d'auteurs qui ont vu l'Épidermophytie plantaire se développer après le contact, des pieds nus, avec la terre.

SUMMARY

The authors were able to isolate from the superficial layers of the soil, *Ctenomyces interdigitalis* (*Epidermophyton interdigitale*, *Epidermophyton* KAUFMANN-WOLF). The morphological macro- and microscopical features of the cultures, as well as those of the ex-

perimental lesions, obtained by inoculation in man and animal, proved that the isolated fungus was really *Ctenomyces interdigitalis* and not *Ctenomyces mentagrophytes* or *Trichophyton* (*Achorion*) *quinckeanum*. The isolated strain caused a dysidrosis of the palm. The patient, a woman 34 years old, infected herself by scooping with her left hand the earth mixed with dead (withered) leaves which were found in a watering pond. The authors did not find, up to now in the literature, any mention about the presence of this dermatophyte in the soil, which would be proved by direct investigation, although its presence in this medium was accepted by those who observed tinea pedis developing after the contact of the feet with the earth.

Bibliographie

1. AJELLO L., Soil as natural Reservoir of Human Fungi. Science, May 18/1956, vol. **123,** no. 3203, pp. 876—879.
2. BRUHNS C. & ALEXANDER A., Handbuch f. Haut u. Geschlechtskrankheiten, Sous la rédaction de Jadassohn, Ed. II, p. **211**, ed. J. Springer, Berlin, 1928.
3. EVOLCEANU R., AVRAM A., ALTERAS I. & ROXIN T., Dermatomicoze, pp.62—63, Editura Medicală, Bucuresti, 1956.
4. EVOLCEANU R. & AVRAM A., Tricofitia spontană a cobaiului. Posibilitatea transmiterii ei la om. Dermato-Venerologia, **2,** 1958, pp. 121—130, Bucuresti.
5. EVOLCEANU R. & ALTERAS I., Le Microsporum gypseum. Quelques observation cliniques et mycologiques concernant ce dermatophyte. Mycopathol. et Mycol. Appl. **X,** 2. 71—82 (30. XII. 1958).
6. EVOLCEANU R. & ALTERAS I., Considérations à propos des caractères mycologiques et pathogéniques du Keratinomyces ajelloi - Vanbreuseghem 1952, saprophyte du sol. Mycopathol. et Mycol. Appl. **XI, 3,** pp. 196—204 (15.X. 1959)
7. EVOLCEANU R. & ALTERAS I., De l'origine saprophytique, tellurique des dermatophytes. Une espèce du genre Aleurisma, saprophyte du sol et agent d'une dermatomycose expérimentale. Mycopathol et Mycol. Appl. (sous presse).
8. FEJÉR E., OLAH D., SZATHMÁRY S., SZODORAY L. & URI J., Orvosi Mykologia pp. 96—98, 148—150, Akadémiai Kiadó, Budapest, 1957.
9. FUENTES R. A. & ABOULAFIA R., Archives of Derm. Syph. Chicago, 1955, **71,** 478. Cité KOCH & RIETH H., Arch. f. klin. exp. Dermat. Bd. **205,** No. 6, 1958, p. 585.
10. KACHKINE P. N., Dermatomikozi, p. 296, Medghiz, 1954, Leningrad.
11. KOCH H. & RIETH H., Endemische Trichophytie bei Meerschweinchen. Arch. f. klin. exp. Dermat. Bd. **205,** no. 6, 1958, pp 577—585.
12. LURIE H. I. & BOROK R., Mykologia (New-York), **47,** 50, 6. 1955.
13. MACKINNON J. E., Ann. Fac. Med. Montevideo **25,** 53 (1940). Cité par KOCH & RIETH. Arch. f. klin. exp. Dermat. **205,** p. 585 (1958).
14. SZATHMÁRY S., Trichophyton fluviale. Arch. f. Dermat. u. Syph. Bd. **181,** Hift 2, p. 192—203 (1940).
15. SIGALOVA E. E., K voprosu ob izmentchivosti dermatofitov (A propos de la variabilité des dermatophytes) Vestnik Venerologhii i Dermatologhii, nr. **5,** p. 12—18, 1951.
16. VANBREUSEGHEM R., Intérêt théorique et pratique d'un nouveau dermatophyte isolé du sol, Keratinomyces ajelloi, gen. nov. sp. nov. Bull. Classe des Sciences, 5-ème série; 1068—1077 (1952).
17. VANBREUSEGHEM R., Les champignons kératinophyles et kératinolytiques. Atti del VI Congresso Internazionale di Microbiologia. Vol. V 236 Roma 6—12 sett. 1953. Sezione XIV Micetti.
18. VANBREUSEGHEM R., Herpes circiné post traumatique, par Trichophyton quinckeanum. Arch. Belges de Dermat. et de Syph. nr. **3,** 1957.

EPIDEMIOLOGICAL ASPECTS OF DEEP MYCOSES IN VENEZUELA [1]

by

HUMBERTO CAMPINS [2]

(14.XII.1959)

Venezuela is located in the tropical zone of the occidental hemisphere inside the first 12° of North latitude. Its territory which is close to one million square kilometers, is formed by a spacious central plain which at the North is surrounded by massive mountains and at the West and Southeast by an extense oceanic coast. Its weather is influenced by the height and other telluric factors and consequently, is variable: hot, cold, cool, wet, and dry. There are also all variations among regions; they can be of poor vegetation, of scarce rainfall, fruitful, dry, and wet. Such variety of conditions is good for the development of fungi and this is proved by the existence of all deep human mycoses and almost all the superficial ones.

With few exceptions all known cases come from central and occidental regions of the northern part of the country, due to the big population and facilities of diagnosis found in those regions. The existence of some of these infections in the southeast part will be shown when they are adequately investigated. The preliminary studies of a work program directed by BALDÓ (1) whose realization has been recently initiated by the Division of Tuberculosis of the Sanitary and Social Assistance in the Oriental zone of the country, suggest the presence of *Histoplasma capsulatum* and other agents of deep mycoses.

The frequent occurrence of some of these infections in the Venezuelan population and the medical and social importance they have for themselves and for the confusion with other nosological entities led us to propose in the Sixth Venezuelan Congress of Medical Sciences held in Caracas in 1955 (2), the creation of a mycological center to study these problems and to give them the solution. BALDÓ considered better and easier the realization of a Commission of Coordination for the national study of the mycoses; its efforts

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[1] Read in the meeting of the International Society of Human and Animal Mycology during the VI. International Congress of Tropical Medicine and Malaria, LISBON, September 5—12, 1958.

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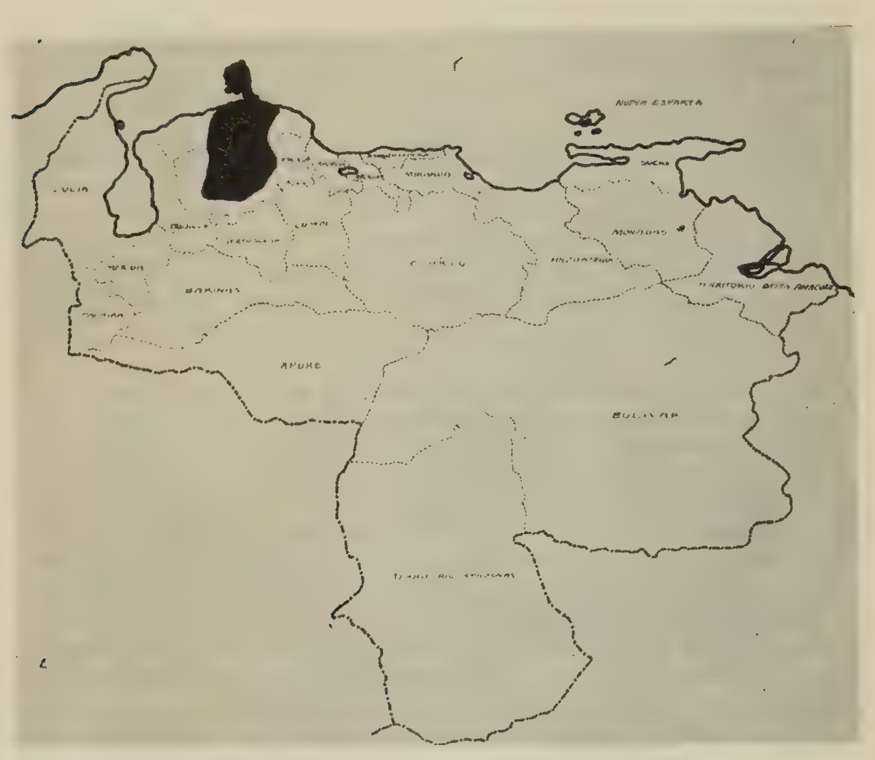

The black section indicates the Endemic zone of Coccidioidomycosis in Venezuela

became reality and today it is to the service of our collectivity. Its development and projects are summarized in a recent publication of this author. (3). Following we will discuss some epidemiological aspects of the studies done in Venezuela on that subject:

COCCIDIOIDOMYCOSIS

There are few cases of this disease known among us, not more than 10, but they have exhibited diverse localizations such as: cutaneous, subcutaneous, pulmonary, and osseous. Except two patients, one from Falcón and other from an unknown place, all have been found to be from Lara State; they were between the ages of 11 months and 60 years and from rural and urban areas. The strains isolated from these cases were the same as those of Coccidioides immitis Rixford and Gilchrist, 1896.

The first endemic zone of the infection was studied by us (4); it comprised the central and northeast region of Lara State and it was more prevalent in the hot, dry areas and also in those whose vegetation was in first place, xerophyte.

The state of Falcón located at the north part of Lara State, has spacious zones, good for the development of Coccidioides immitis. This characteristic helped us to suppose that there was Coccidioidomycosis there. Recent investigation about skin sensibility to coccidioidin carried out in 300 inhabitants of several regions of these States by Zirit and col. (5), gave 11.3% positive proofs being more prevalent in those people living in dry places. These results gave the first proof of our supposition. The definitive confirmation was given by a patient who had always lived in the State of Falcón. (6).

Same kind of studies done by Guillén (7) and Moncada and col. (8) in people resident in Mérida, gave a very low percentage of positivity to coccidioidin. Convit (9) taking advantage of the extensive chain of service of the Lepra Division which belong to the Minister of Sanitary and Social Assistance has initiated a survey which will be carried out in the whole country. Until now, he has practiced cutaneous tests with coccidioidin in three groups, each one of one thousand persons in the rural areas of Bolívar, Zulia, and Lara States obtaining insignificant per cents in the two first states while in the third one the results were of 16.2%. The persons tested were residents of Urdaneta District which is comprised in the area determined by us. Consequently, the endemic Venezuelan areas of Coccidioidomycosis encompass the central and north-west regions of Lara State and the north and central ones of Falcón State. (See map).

HISTOPLASMOSIS

The opportunity to study a group of 16 persons which was exposed to the inhalation of *Histoplasma capsulatum* in the same place and under the conditions, and of which 12 suffered primary Histoplasmosis of the lungs (10) permitted us to obtain information of epidemiological interest.

We observed that those whose intradermoreaction to Histoplasmin was positive before the exposure to the fungi did not give further clinical manifestations of infections. The value of the positive proof as an expression of resistance to the infection is confirmed, but there is still an interesting question. In effect the specificity of Histoplasmin is not so high as that of Coccidioidin and in certain cases the reaction to Histoplasmin may be a consequence of infection by *Coccidioides immitis* or other fungi. Does this also mean that such organisms are protected against *Histoplasma capsulatum*?

In a person whose cutaneous reaction to Histoplasmin was negative immediately before the exposure to the fungi and whose symptoms of infection were apparently 16 days later, the initiation of cutaneous sensibility to the antigen after 18 days of contact could be observed.

The period of incubation of 12 patients, was between 8 and 16 days and there was no relation between the variation of this period and the severity or benignity of the infection. The clinical picture was very similar in all cases but the intensity of the symptoms and the duration of the sickness were different, which led to classify these infections in benign and severe forms. All pulmonary X-rays showed enlargement of the hilar lymph nodes the severe cases showed numerous modular infiltrates in both lungs, due to the inhalation of the fungi.

These persons were infected in a cave located in the state of Lara; from its soil *Histoplasma capsulatum* was isolated. BELLARD and col. (11) informed about other patient who got the infection in a cave located in Miranda State. Later MONTEMAYOR and col. (12), studying the caves that exist in Venezuela, isolated *Histoplasma capsulatum* from the cave mentioned above and from others located in the country. The most important cave of Venezuela is the one called "Cueva del Guácharo" which is inhabited by birds named Guácharos (*Steatornis caripensis*, HUMBOLDT, 1814) and it is located in the oriental part of Venezuela. Soil samples of several sections of this cave were studied by AJELLO from North America (13), and by us, but the fungi could not be found in the samples used. Although near this cave and in other ones located in the island of Trinidad, inhabited by Guácharos, AJELLO (14) has proved the existance of *Histoplasma capsulatum*. BORELLI (15) isolated a strain of the same species among 18 samples of soil taken from places around Caracas. All the strains isolated from patients and soils were identical with *Histoplasma capsulatum*, DARLING 1906.

The cases of Histoplasmosis, which are around 20, and the studies of skin sensibility carried out by CAMPINS (16), POLLAK (17), GUILLÉN (7), and MONCADA and col. (8) show that *Histoplasma capsulatum* has wide distribution in Venezuelan territory. In one of the cases, the infection took place in the laboratory. Almost all the patients were males, between the ages of 7 months and 53 years.

PARACOCCIDIOIDOMYCOSIS

The cases of this mycosis are numerous among us. They are more than 200 and the strains isolated belong to the species type *Paracoccidioides braziliensis*. (SPLENDORE 1912), ALMEIDA 1930. The patients came from central and occidental states of the country and most of the time, they were farmers and inhabitants of rural areas. However, 12 of 23 cases published by BALDÓ (18) and 2 of 8 contained in one of our informations (2) were from the city. The greater incidence of the clinical pictures has been observed in adults, being prevalent in the fourth decade of life. Only two cases affecting children have been published (19), (20). The occurrence of the sickness in the male sex is almost absolute. It is between 90 and 100% in the diverse publications.

CHROMOBLASTOMYCOSIS

The known cases of Chromoblastomycosis are close to 100. There are curious factors to mention. One is, the frequent localization of the lesions on upper limbs and the face, with a clinical aspect often different from those producing warts. Other factor is the high number of women affected which can be explained by the active participation of our peasant women in the task of her envirement and the existence of thorny plants in those regions where the greatest number of cases come from; all these circumstances increase the means of inoculation. Lastly, it is good to mention that the agent frequently isolated is a distinct species from those commonly found in other countries. TREJO, based on the study of four Australian strains of POWELL and three of the many isolated by us (21), proposed the designation of *Cladosporium carrioni*.

A cutaneous wound caused by vegetable particles followed by the progressive development of lesions in many cases validates the concept that those fungi have also a parasitic or saprophytic life on certain vegetables and they constitute a source of human infections.

The extreme ages of Venezuelan cases are between 16 and 80 years, being more prevalent between 20 and 60 years.

The sickness has been proved in the inhabitants of the central and occidental areas of the country, whose territories have variable climatic and phytographic conditions. The majority of infected people were residents of Lara State when the sickness began and almost all of them lived in dry areas with rare precipitation and abundant xerophyte vegetation in which thorny plants were predominants.

ACTINOMYCOSIS AND NOCARDIOSIS

About 30 human cases of this sickness caused by Actinomycetes have been published in our country. There are clinical reasons to suspect that in some of the patients and in bovine cases, the causative agent was *Actinomyces bovis*. The presence of *Actinomyces bovis* in human cases has been recently also confirmed (22) (23).

The agents frequently isolated have been *Actinomyces* or *Nocardia brasiliensis* (LINDENBERG 1909). Three strains have corresponded to *Actinomyces* or *Nocardia madurae* (VINCENT 1894) and two have been identified as *Actinomyces* or *Nocardia asteroides* (2).

Almost all patients were adults, farmers, males showing the best decade of their life. The occidental and central regions of the country were the living places of these people but most of them were from Lara State.

MADUROMYCOSIS

Twelve of the fourteen cases published in which the diagnosis of Madumroycosis can be accepted, had black granules and two cases had white ones. One of these were attributed to the genus *Aspergillus* and the culture of the other did not show pathogenic fungi. Most of the patients who showed black granules were affected by *Madurella grisea*, MACKINNON, FERRADA & MONTEMAYOR, 1948. Two strains of *Madurella mycetomi*, BRUMP 1950, have been also isolated.

All patients studied were males between the ages of 14 and 60 years, working in agriculture. The beginning of the infection was related to the penetration of vegetable fragments at the site of the lesion. Eleven patients resided in Lara State, when the pathological process began.

OTHER DEEP MYCOSES

The Venezuelan incidents of Cryptococcosis, formed by a dozen cases, do not permit to get an important epidemiological information. Patient of both sexes, at ages of full activities, living in urban and rural areas and with diverse occupations were involved. Sporotrichosis exists in the whole country and surely it is more frequent than the cases published.In some of these there is a clear relation between the beginning of the sickness and a wound caused by vegetable fragments.

There are also numerous cases of Rhinosporidiosis which come from the central and occidental region of the country which have effected people of both sexes between ages of 11 and 55 years, from rural and urban residences and with diverse occupations.

There is just one proven case of Gilchrist disease (24). The patient had lived in Venezuela and had been for long time in Valencia, a city located at the north central area of the country. Only buccolaryngeal lesions were found in the patient.

SUMMARY

The tropical situation of Venezuela is a favorable condition to the development of fungi and contributes to the varied pathology in which numerous species are the agents. All the known deep human mycoses have been found on the Venezuelan territory. Studies of skin sensibility to histoplasmin and the analysis of the origin of cases of histoplasmosis and paracoccidioidomycosis indicate that both are very frequent in our country and are found preferentially in humid places of leafy vegetation, while coccidioidomycosis is limited to a dry area with predominant xerophyte vegetation and scarce rain fall, located in the northwestern region of the country, near the central North. *Histoplasma capsulatum* has been isolated from soils, among them the one of a cave where several excursionists had acquired the infection. It has been possible to pinpoint in these cases of primary pulmonary infection that the incubation period oscillated between 8 and 16 days and in one of

them the appearance of the skin sensitivity to histoplasmin could be observed 18 days after the exposure to the fungus.

In the most part of cases of chromoblastomycosis the agent is a different species from those frequently found in other countries and it seems to be abundant in dry regions with xerophyte vegetation.

A *"National Coordinating Commission for the Study of Mycoses"* has been established in order to facilitate the elucidation of diverse problems that mycoses brought up and to stimulate the interest in medical mycology in the country.

ZUSAMMENFASSUNG

Die tropische Lage von Venezuela schafft eine günstige Bedingung für die Entwicklung von Pilzen und trägt zu einer vielfältigen Pathologie bei, in welcher eine grosse Anzahl dieser Pilze die Ursache ist. Alle bekannten, systematischen, tiefen Mykosen sind im Gebiete von Venezuela gefunden worden. Untersuchungen der Hautempfindlichkeit gegen Histoplasmin und die Analyse der Herkunft der Fälle von Histoplasmosis und Paracoccidioidomycosis zeigen, dass beide Infektionen im Lande weit verbreitet sind und dass sie vorzugsweise in feuchten Gegenden mit Laubvegetation vorzufinden sind, während Coccidioidomycosis in trockenen Gegenden mit einer vorzugsweise xerophyten Vegetation, mit mangelndem Regen, im Nordwesten des Landes, nahe dem Centralen Norden vorkommt. *Histoplasma capsulatum* ist von verschiedenen Böden gezüchtet worden, unter anderem, im Boden einer Höhle, wo mehrere Ausflügler die Infektion erworben haben. Es war möglich in diesen Fällen primärer Lungeninfektion festzustellen, dass die Inkubation zwischen 8 und 16 Tagen liegt, und dass in einem der Fälle die Erscheinung der Hautsensibilität zu Histoplasmin 18 Tage nach der Infektion erfolgte.

In den meisten Fällen von Chromoblastomykosis ist die ursächliche Pilzspecies eine andere, als diejenige, die in anderen Gegenden gefunden worden ist. Sie ist reichlich vorhanden in trockenen Gegenden mit einer xerophyten Vegetation.

Eine *"Nationale Koordinierende Kommission für die Untersuchung von Pilzerkrankungen"* ist gegründet worden, um die Aufklärung der mannigfaltigen Probleme, die die Mykosen hervorgerufen haben, zu erleichteren und um das Interesse für die Medizinische Mykologie im Lande zu fördern.

Bibliography

1. Baldó, J. A.: Personal communication.
2. Campins, H.: 1955. Mycosis profundas endémicas en Venezuela. Memor.VI Cong. Venez. de Cienc. Méd. 18—26 Nov. **5**: 2786.
3. Baldó, J. A.: 1958. The Study of Mycoses on a nation Wide Scale in Venezuela. Bull. Int. Soc. Hum. Anim. Mycol. **3**: 13.

4. Campins, H.: 1950. Coccidioidomicosis: Un nuevo problema de salud pública en Venezuela. Rev. Sanid. As. Soc. Caracas **15**: 1.
5. Zirit, R., Graterol, C. A. & Fernandez, A. J.: 1957. Contribución al estudio de la Coccidioidomicosis en el Estado Falcón. Rev. Col. Méd. Edo. Falcón. **6**: 2.
6. Graterol, C. A.: Personal communication.
7. Guillén, C.: Sensibilidad cutánea a la Histoplasmina, coccidioidina y tuberculina. Investigación en grupos seleccionados. Tesis doctoral. Univer. de Mérida, 1955.
8. Moncada, F., Sequera, H. & Gabaldón, A.: 1957. Encuesta epidemiologica de tuberculina, coccidioidina e histoplasmina en 1,000 personas del Estado Mérida. Rev. Coleg. Méd. Edo. Mérida. **7**: 377.
9. Convit, J.: Personal communication.
10. Campins, H., Zubillaga, Z., Gómez Lopez, L. & Dorante, M.: 1956. An epidemic of histoplasmosis in Venezuela. Amer. J. trop. Med. Hyg. **5**: 690.
11. Bellard, E. P., Briceño, L., Pardo, I., Requena, A & Pollak, L.: 1956. La Histoplasmosis en Venezuela. Observaciones. Gac. Méd. de Caracas, **63**: 191.
12. Montemayor, L., Heredia, B. & Lobig, I.: 1957. Técnicas para aislamiento de Histoplasma capsulatum de muestras de tierra y hongos saprofitos del suelo. Bol. Venezol. Lab. Clínico. **7**: 139.
13. Ajello, L.: Personal communication.
14. Ajello, L.: Personal communication.
15. Borelli, D.: 1957. Micosis en Venezuela. Rev. Fac. Med. Caracas **3**: 31. Acta Cientif. Venezol. 1937.
16. Campins, H. & Scharyj, M.: Investigacion de la sensibilidad cutanea a la Histoplasmina en Venezuela.
17. Pollak, L.: 1953. Histoplasmosis en Venezuela. Ensayo epidemiologico. Rev. Sanid. As. Soc. Caracas. **18**: 569.
18. Baldó, J. A.: 1953. Paracoccidioidomicosis pulmonar. Rev. Sanid. As. Soc. Caracas. **18**: 163.
19. Potenza, L., Lares, C. & Feo, M.: 1953. Paracoccidioidosis infantil en Venezuela. Arch. Venezol. Pueric. y Pediatría. **16**: 7.
20. Barnola, J., & Alvarez, P. J.: 1956. Paracoccidioidosis de forma ganglionar, infantil. III Jorn. Pueric. y Pediat. Maracaibo.
21. Campins, H. & Scharyj, M.: 1935. Cromoblastomicosis. Comentarios sobre 34 casos con estudio clínico, histológico y micológico. Gac. Med. de Caracas. **61**: 127.
22. Velutini, L., Borelli, D. & Estrada, E.: 1957. Micetoma cervico-facial por Actinomyces bovis en mujer con síndrome de Cushing. Rev. Centro Méd. de Caracas. **2**: 9.
23. Alarcón, C. J., Convit, J. & Borelli, D.: 1957. Micetoma del brazo por Actinomyces bovis. Dermat. Venezol. **1**: 87.
24. Polo, F.J., Brass, K. & Montemayor, L.: 1954. Enfermedad de Gilchrist en Venezuela. Rev. Sanid. As. Soc. Caracas. **19**: 217.

MOUSE FAVUS DUE TO TRICHOPHYTON QUINCKEANUM (ZOPF) MACLEOD & MUENDE: A REAPPRAISAL IN THE LIGHT OF RECENT INVESTIGATIONS. IV

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(23.X.1959)

CULTURAL FEATURES OF T. QUINCKEANUM.

The following remarks, except where specified, apply generally to all the strains of *T. quinckeanum* which have been cultured in this Department including strain 14 kindly supplied by Mr. AUSTWICK of the Veterinary Laboratory at Weybridge in Surrey.

Primary cultures of strains isolated here were incubated at 27°C. on the standard Sabouraud agar media: Conservation medium (3% peptone) and Test medium (4% glucose and 1% peptone); and also on honey-peptone agar (5% honey and 1% peptone). The peptone used was "Oxoid" bacteriological peptone; the agar concentration was 2%. To counteract bacterial contamination, mixed antibiotic solution, containing Streptomycin and Penicillin, was added to the medium by the technique developed in this Laboratory by FORSTER (13) to give a final concentration of 40 units of Streptomycin per ml. and 20 units of Penicillin per ml.

Macroscopic features of cultures on 5 ml. slopes in 1.5 cm. diam. test-tubes plugged with absorbent cotton wool.

On conservation agar, growth was usually visible at 3 days, and within 5 days, had produced a more or less well defined central white floccose nodule at the surface of the medium: from this nodule the surface mycelium spread radially. At about 14 days, the surface growth had become matted down, was irregularly depressed, and furrowed or crimped or denticulate at the margin. (Plate I fig. 1b and fig. 2). Its surface colour was snow-white. On the reverse side, the growth gradually became tinged a pale yellow.

On test medium, growth was generally visible in about 3 days, and within 7 days had formed a central white nodule surrounded by a radiating fringe which rapidly increased in density. At 14 days, the surface growth was still more or less floccose and did not exhibit the irregular depressions seen in the conservation medium cultures, while its margin was uniform or denticulate; the colour of this surface growth was snow-white but gradually became suffused with a pale buff tinge; the reverse was in various shades of yellow. (Plate I fig. 1c).

On honey-peptone agar (Plate I fig. 1a) the growth usually resembled that on the glucose test medium, but occasionally, during the first few days, was conspicuous for its development of a white compact nodule at the surface of the medium surrounded by a disproportionately narrow radiating fringe. However, as growth proceeded, these features became less definite and these cultures closely resembled those on the Sabouraud test medium.

Production of pigment in the cultures.

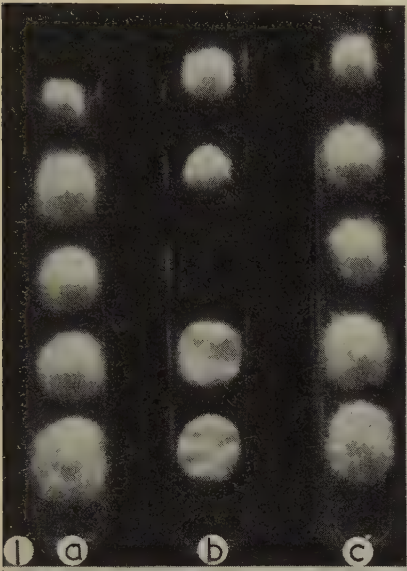
Some authors, notably, BODIN (1) VANBREUSEGHEM (2) and BLANK (3) drew attention to the production of a purplish-red or violet pigment on the reverse of the cultures of *T. quinckeanum*. In the strains studied here, this pigment was conspicuous only in 2 strains and even then was not a constant feature. These strains were the last two isolated, viz. 15 and 16. This pigment occurred in patches on the reverse side and was sometimes associated with a reddish-brown pigment. In individual slopes some of the colonies exhibited some "vinaceous fawn" (RIDGWAY, 4) pigment in their surface growth at 27° C. Besides these two strains, strain 12 and strain 13a exhibited some small patches of vinaceous fawn in their surface growth and on their reverse in individual slopes of test medium. This pigment did not develop in any of the other strains even when they were incubated for 7 days at 27°C and then transferred to the 37° C incubator.

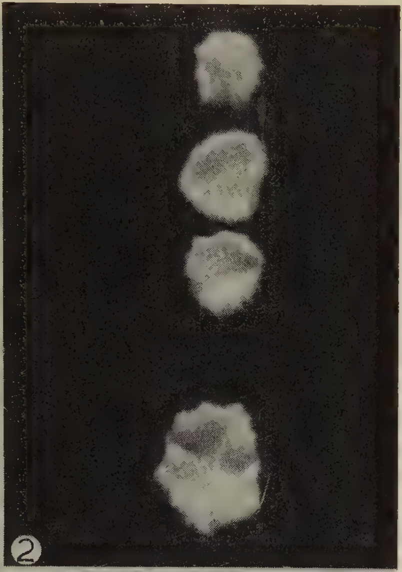
Petri dish cultures at 27° C.

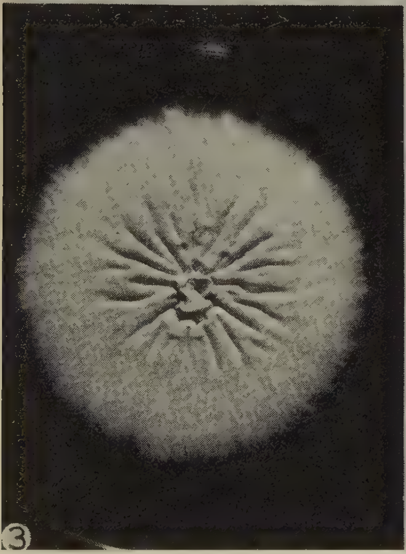
On both standard Sabouraud media in Petri dishes the various strains showed, in due course, folding at the centre and furrowing in a radial pattern. On tomato juice peptonised milk (Oxoid) agar radiating folds were also present. (Plate I figs. 3 and 4).

Microscopic features of cultures on standard Sabouraud media and on natural media.

Almost all the various kinds of microscopic structure which have been described from cultures of dermatophytes were observed in some or all the strains studied in Leeds. They were present on several







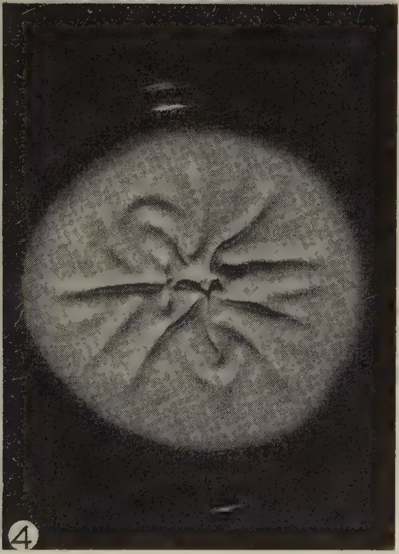


PLATE I

Fig. 1. Primary cultures of *T. quinckeanum* strain 13c; a) honey-peptone agar, b) Sabouraud conservation medium, c) Sabouraud test medium. 9 days old; approx. ¾ nat. size.

Fig. 2. Primary culture of *T. quinckeanum* strain 11 on Sabouraud conservation medium; approx. nat. size.

Fig. 3. Second subculture of *T. quinckeanum* strain 16 on tomato juice plus peptonised milk agar; 16 days old; approx. 3/4 nat. size.

Fig. 4. First subculture of *T. quinckeanum* strain 12 on Sabouraud test medium; 19 days old; approx. 1/2 nat. size.

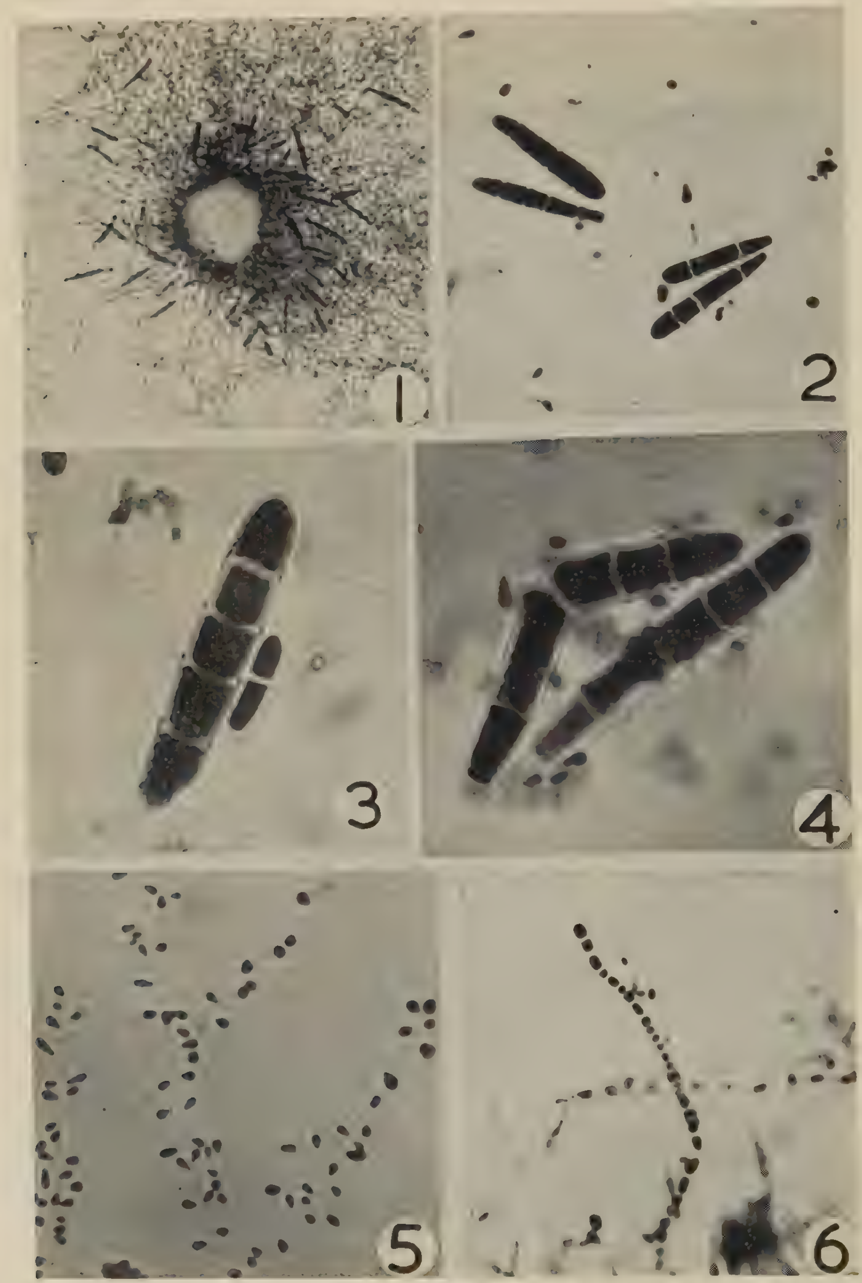


PLATE II

Fig. 1. Centre of macroconidial production; 7-day old culture of strain 12 on bran agar; × 120.

Fig. 2. Macroconidia showing variation in shape; 7-day old culture of strain 16 on 3% peptone-in-broth agar; × 514.

Fig. 3. A large and a small macroconidium; 7-day old culture of strain 16 on 3% peptone-in-broth agar; × 1300.

Fig. 4. Fragmenting macroconidium; 7-day old culture of strain 13c on bran agar. × 1300.

Fig. 5. Microconidia u,en thyrse"; 25-day old cover-slip drop culture of strain 13b on tomato juice plus peptonised milk agar. × 514.

Fig. 6. Arthrospores; 7-day old culture of strain 13a on 3% peptone-in-broth agar. × 514.

or all of the culture media. Those observed so far were: — arthrospores, chlamydospores, nodular structures, spiral hyphae, microconidia and macroconidia.

Arthrospores.

Seen in strain 13a on 3% peptone-in-broth agar. They were somewhat rectangular or oblong and thick-walled, and formed a linear series. (Plate II, fig 6.) They were somewhat reminiscent of the arthrospores of *Trichophyton verrucosum* var. *discoides* and of *T. mentagrophytes* var. *granulosum* in cultures on Sabouraud conservation medium incubated at 37°C.

Chlamydospores.

Seen in a number of strains, on lengths of straw, tomato juice plus peptonised milk agar, 2% malt agar cover-slip cultures and on 3% peptone-in-meat-broth gelatine and 3% peptone-in-meat broth agar. They were more or less spherical or elliptical, and thick-walled when fully developed, measuring up to 15 μ in their widest diameter in strain 12 on straw at 21 days, and were terminal, intercalary or in chains. (Plate IV, fig. 2)

Nodular structures.

Seen in all strains within the matrix of agar-containing media and in 3% peptone-in-meat broth gelatine. They varied from short or long intertwining hyphae (Plate IV, fig. 3) to stout multicellular hyphae interwoven in dense knots. (Plate IV, fig. 5).

Spiral hyphae.

Observed occasionally in strains 7, 8, 12, 13a, b, and c on one or several of the following media: — 2% maltose agar as used by RIVALIER (5) in slide cultures; honey-peptone slopes, tomato juice plus peptonised milk agar cover-slip culture drops, carrot blocks and on potato-carrot agar slopes (strain 12). They were found usually only after much searching, probably owing to the very numerous microconidia which were present, but also occasionally by chance.

They were mainly unbranched, but were occasionally branched, and their stalks appeared to be disproportionately long (Plate IV, fig. 1) N.B. (Spiral hyphae were observed in this dermatophyte by LANGERON & MILOŠEVIĆ (6) in cultures on straw.)

Microconidia.

Observed on all media in cultures which had not become pleomorphic, and were produced in enormous quantities. They usually occurred "en thyrse" but occasionally also on branching systems which closely resembled those generally described by the term "en grappe". They were oval to pyriform, or elongate with more or less parallel sides, truncate at the base and thin-walled and smooth. Their dimensions in a number of strains grown on tomato juice plus peptonised (Oxoid) milk agar at 27° C were recorded at 10 days when mounted in water, and are shown in table II.

TABLE I.

Macroconidia at 10 days on tomato juice plus peptonised milk agar. 50 macroconidia of each strain measured unstained in water.

Strain number	Length		Breadth	
	Max.	Min.	Max.	Min.
7	45.0 μ	12.3 μ	9.5 μ	3.8 μ
8	53.2 μ	7.6 μ	9.5 μ	3.8 μ
11	58.9 μ	13.3 μ	11.4 μ	3.8 μ
12	56.1 μ	12.3 μ	7.5 μ	2.8 μ
13c	76.0 μ	14.2 μ	9.5 μ	3.8 μ
14	49.4 μ	13.3 μ	9.5.μ	3.8 μ
15	41.8 μ	13.3 μ	8.5 μ	4.7 μ
16	53.2 μ	11.4 μ	11.4 μ	3.8 μ

TABLE II.

Microconidia at 10 days on tomato juice plus peptonised milk agar. 50 microconidia of each strain measured unstained in water.

Strain number	Length		Breadth	
	Max.	Min.	Max.	Min.
7	9.5 μ	3.8 μ	3.8 μ	1.9 μ
8	7.6 μ	2.8 μ	4.7 μ	1.9 μ
11	6.6 μ	3.8 μ	4.7 μ	1.9 μ
12	7.6 μ	3.8 μ	3.8 μ	0.9 μ
13c	6.6 μ	3.8 μ	3.8 μ	1.9 μ
14	7.6 μ	3.8 μ	4.7 μ	2.8 μ
15	7.6 μ	3.8 μ	4.7 μ	1.9 μ
16	6.6 μ	2.8 μ	3.8 μ	1.9 μ

Macroconidia.

On the standard media, macroconidia were not seen in any of the strains studied here. A few only were observed in strain 12 in a 10-day old honey-peptone agar slope at 27 C. On certain natural media

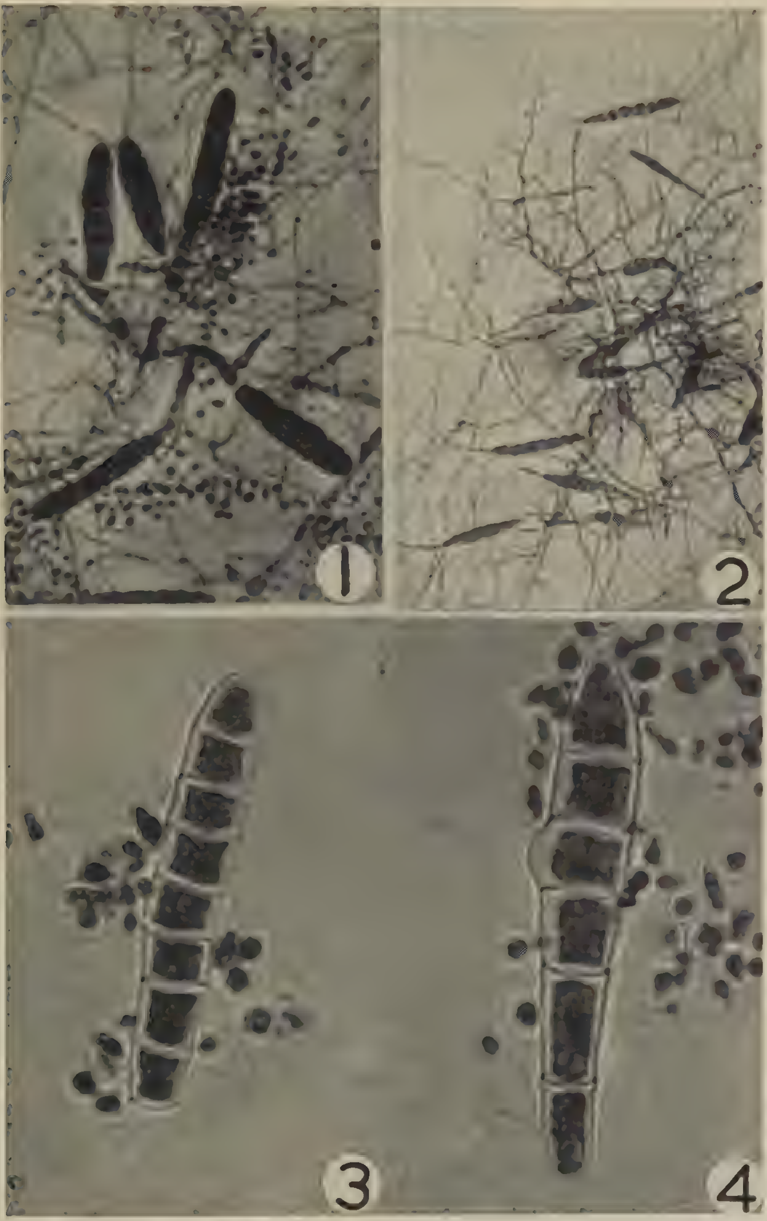


PLATE III

Fig. 1. Clump of pedicellate macroconidia; 6-day old culture of strain 12 on 2% malt extract agar slide film. × 514.

Fig. 2. Macroconidia with filiform appendages; 4-day old culture of strain 6 on wheat grain section mounted on cover-slip. × 200.

Fig. 3. Macroconidium with blunt tip showing space between wall and cytoplasm; 17-day old culture of strain 13c on tomato juice plus peptonised milk agar. × 1300.

Fig. 4. Macroconidium with pointed apical cell and lateral bulge also showing space between wall and cytoplasm; 17-day old culture of strain 13c on tomato juice plus peptonised milk agar. × 1300.

they were, on the contrary, produced in great quantity within a few days of the medium being inoculated. These natural media included: — wheat grains, bran agar (bran 7.7%, pH 6.1), tomato juice agar (Oxoid) pH 6 (strains 8, 12, 13c and 15) and tomato juice plus peptonised milk agar pH 6, as well as 3% peptone-in-broth agar pH 5.4. They were produced in lesser quantities on 3% peptone-in-broth gelatine (which was QUINCKE'S medium) and on other natural media such as straw, carrot blocks and 2% malt extract agar. On bran agar slopes at 27°C they developed in 4—5 days, and on tomato juice plus peptonised milk they were present at 5 days. On slopes smeared with inoculum, they usually originated from centres of dense mycelial growth, forming radiating clumps (Plate II fig. 1), a feature already remarked by BLANK (l. c.). They were generally pedicellate (Plate III. fig. 1.) but at times almost sessile. They varied much in size and shape in any one strain (Plate II figs. 2 & 3.). Their max. and min. length and breadth are recorded in table I for a number of strains grown on tomato juice plus peptonised milk agar slopes at 27°C. the measurements having been recorded at 10 days from material mounted in sterile water, using a micrometer scale in a 4 X ocular lens and a 1/6 objective lens. Viewed with a 1/12 objective lens and 10 × ocular and mounted in lacto-phenol solution containing Cotton blue (.05%) they were seen to be clavate, parallel - sided or spindle-shaped, while others still were otherwise variously shaped: curved or sinuate or inflated laterally, (text-fig. 1 a and b and Plate III fig. 4.). They were usually blunt-ended, but occasionally, more or less pointed, and were multilocular (2—7, occasionally 8 locules). They were delimited by a relatively thin wall (as compared with *M. canis*) which resembled in degrees of thickness that found in the macroconidia of varieties of the *T. mentagrophytes* group viz. vars. *granulosum, asteroides* and *persicolor*. Many were surmounted at their distal end by a continuous or septate filiform appendage which contained protoplasm and which was separated from the apical cell of the macroconidium by a septum. (Plate III fig. 2 and text-fig. 1b and d) (Such filiform appendages were evidently designated by QUINCKE (7) when he described the macroconidia of α - pilz as having their distal ends drawn out into long narrow points.). They were also observed by MEWBORN (8). They are difficult to interpret, but are not peculiar to this dermatophyte, for they have been observed in other species of *Trichophyton* and *Microsporum* as well, notably, in *M. gypseum* by VANBREUSEGHEM (2) and by the present author (unpublished data) in *T. persicolor*. They are conspicuous, particularly in primary cultures where they may be observed on young macroconidia which are still attached by their pedicels. In mature cultures and in subcultures, similar structures may be germination hyphae and occasionally some with branches suggested this. In any case, although they attract attention, they are of no taxonomic significance since they occur in a number of dermatophytes. On bran

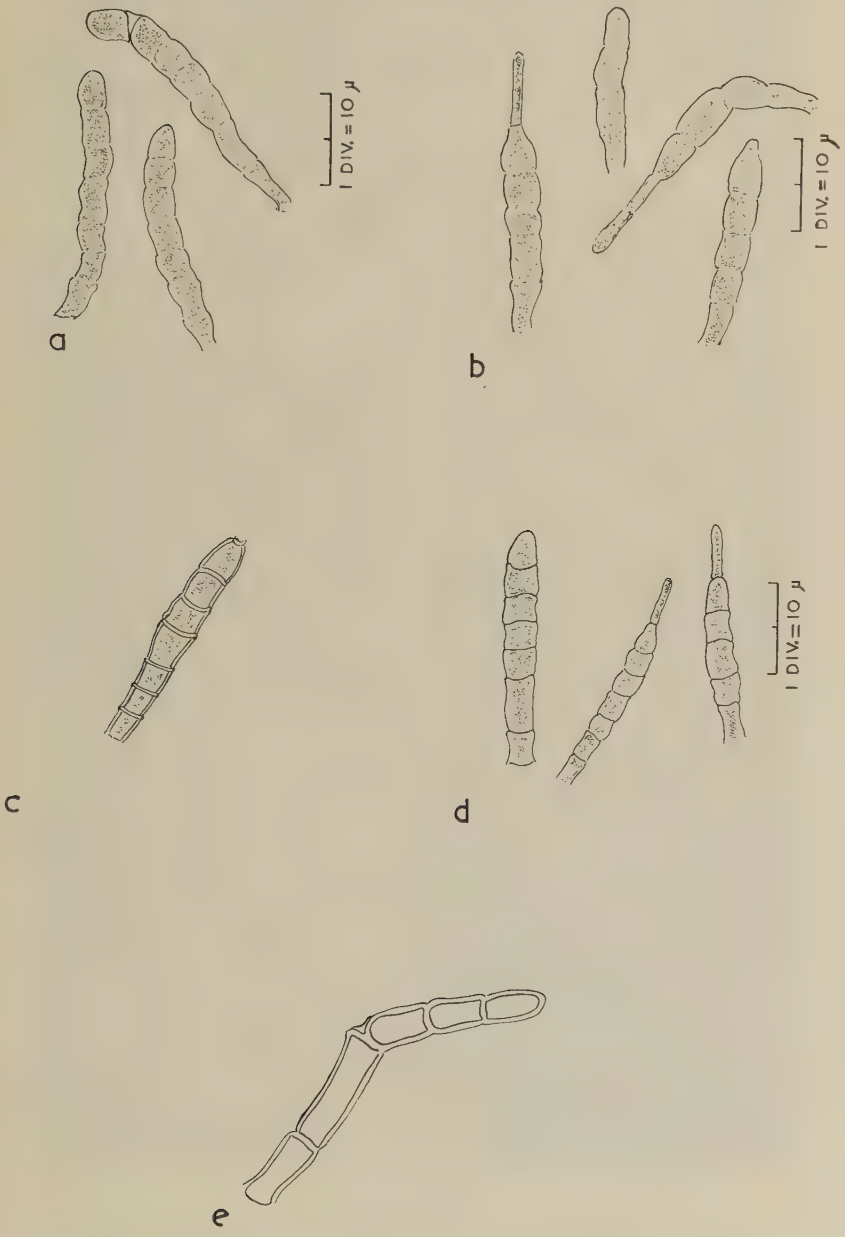


Fig. 1.

a. Macroconidia of strain 13c from 7-day old culture on bran agar. Camera lucida. × 650.

b. Macroconidia of strain 13c from 6-day old culture on tomato juice plus peptonised milk agar. Camera lucida. × 650.

c. Tracing of macroconidium shown in plate IV fig. 4. showing structural details in diagrammatic form. × 650

d. Macroconidia of strain 13c from 17-day old culture on tomato juice plus peptonised milk agar. Camera lucida. × 650.

e. Diagrammatic sketch of macroconidium shown in Plate II. fig. 4. illustrating method of separation of loculi.

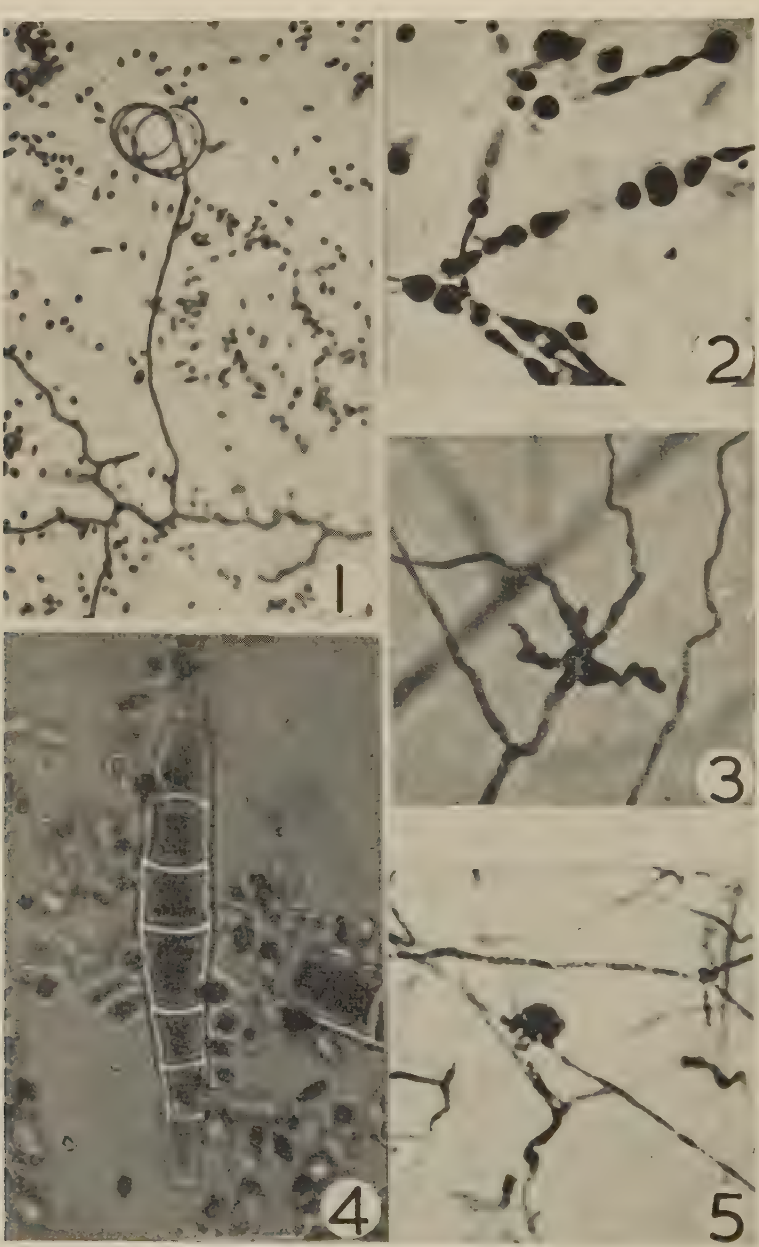


PLATE IV

Fig. 1. Spiral hypha; 10-day old culture of strain 13b on tomato juice plus peptonised milk agar. × 514.

Fig. 2. Chlamydospores; 21-day old culture of strain 12 on straw. × 514.

Fig. 3. Nodular organ; 5-day old culture of strain 13c on 2% malt extract agar slide film. × 514.

Fig. 4. Macroconidium showing space between refractive wall and stained cytoplasm; 17-day old culture on tomato juice plus peptonised milk agar. × 1300.

Fig. 5. Nodular organ; 7-day old culture of strain 11 on 3% peptone-in-broth agar. × 514.

agar, most of the foregoing features were observed, but many of the macroconidia reached greater lengths, those with 7 and 9 loculi being more numerous than on tomato juice plus peptonised milk agar.

Details of macroconidial structure.

Since recent authors, notably BLANK (l. c.) and VANBREUSEGHEM (l. c.) have paid special attention to the features of the macroconidia of this dermatophyte in discussing its taxonomic status, it is appropriate to record the following details as observed by the present author in strains studied here:

The parietal and transverse walls were of approximately equal thickness and any increase in thickness in them which may have taken place with age was not perceptible, the apparent thickness observed in older macroconidia being due to retraction of the cytoplasm from the parietal and transverse walls, leaving a space around it which, on cursory examination, appears to form part of the wall of the loculi. This feature may be seen distinctly only with judicious manipulation of the condenser and iris diaphragm, using a 1/12 obj. lens and a 10 × ocular lens to examine macroconidia mounted in lacto-phenol containing Cotton blue (.05%). It may then be seen that the parietal and transverse walls are refractive whereas the space between them and the cytoplasm is dull. These features are illustrated in the accompanying photomicrograph and a tracing made from it (Plate IV, fig. 4. and text-fig. 1c.).

In many cases where the macroconidium had become partly fragmented, it was observed that the transverse walls of contiguous loculi had split along part or the whole of their length and that when the daughter walls thus formed had partly separated their opposing surfaces had become convex: a process which seemed to commence at a point on the periphery where a triangular channel was already present. This was usually discernible as a dark dot at either end of the transverse partition between the two loculi when the macroconidium was examined in optical section (Plate II, fig. 3). It was also observed in the case of some partly disrupted macroconidia that there was present a very fine membrane which spanned the peripheral side of the cleft which resulted from the initial separation in part of the daughter transverse walls (Plate II fig. 4 and text-fig. 1e). The remains of this membrane were observable usually as a minute tag on either side of the separated end of the loculi.

Comment.

In this author's opinion most of the above-mentioned structural details of the macroconidia of this dermatophyte are probably common to most species of dermatophyte which produce these conidia, but are recorded here because they have not been noted elsewhere and for the reason already expressed at the commencement of the first paragraph dealing with these details.

Conclusions.

The macroscopic features of the cultures of this dermatophyte are, when considered collectively, sufficiently distinct and characteristic when it is grown on the standard Sabouraud culture media. The microscopic features of cultures on these media are, on the contrary, not sufficiently distinct from those found in other species to warrant specific significance. Even when full morphological development is realised on certain natural media the additional structures thereby produced are in the main so like those found, for instance, in the *Trichophyton mentagrophytes* group, that they cannot be regarded as specifically different. It thus appears that, on the basis of its microscopic morphology in culture, this dermatophyte ought to be assimilated with the *Trichophyton mentagrophytes* group.

GENERAL DISCUSSION AND GENERAL CONCLUSIONS

It is the hope of the present author that the foregoing data resulting from the study of strains of *T. quinckeanum*, isolated chiefly from patients in this region, will enable taxonomists to determine more precisely the taxonomic status of this dermatophyte. The body of research which forms its historical background has been inconclusive in this respect owing mainly to the incomplete morphological picture available to the majority of authors who have studied the subject since QUINCKE published his discoveries. In the first place, QUINCKE (l.c.) clearly described both the macroscopic and microscopic features of α - Pilz in culture. He stated that, on 3% peptone-in-meat broth gelatine at room temperature, the surface growth was snow-white and the reverse of this growth was yellow to yellowish brown. These features were well illustrated by him in colour in his Plate I figs. 5 and 6. On examining these cultures he observed: "rundliche und längliche Sporen von 3—6 μ Durchmesser . . ." as well as: Makrogonidien, endständige spindel-förmige Gebilde (von 48—70 μ Länge) mit glänzender doppelt contourierter Wand und gleich-beschaffenen Querscheidewänden, durch welche die Spindel in 4—7 Fächer getheilt wird;" and describing them in further detail, he continued: ,,Zuweilen hatte es den Anschein, als ob in jedem dieser Fächer sich eine ovale oder oblonge glänzende Spore bilde; nach Analogie anderer Pilze würde das ganze Gebilde als eine mehrzellige Spore anzusehen sein.) Das vordere Ende dieser Makrogonidien ist bald stumpfkeulenförmig, bald (und zwar häufiger an den Luftästen) zu einer langen schmalen Spitze ausgezogen. (Fig. 4. Taf. II. III)." It is almost superfluous to state that these findings have been confirmed in their essential features by the relevant data obtained by the present author. To be sure, QUINCKE appears to have omitted to measure smaller macroconidia which were most probably present in his cultures, but other authors who have given measurements for the macroconidia of *T. quinckeanum* also seem to have ignored them. Perhaps the reason for this has been that they considered such small forms to be abnormal or immature. There is no doubt, at any rate, that they were of quite frequent occurrence in the author's strains, and, in recording their dimensions, he has followed the example of EMMONS (10).

It is strange that SABOURAUD (11) should have given an almost complete description of this dermatophyte supported by illustrations, and yet have omitted to acknowledge QUINCKE'S original description of his α-Pilz. Stranger still that he should have acclaimed BODIN as the first authentic describer of the true etiologic agent of Mouse Favus without commenting on the discrepancies between his own and BODIN'S descriptions of the same strain. VANBREUSEGHEM (2) has already alluded to this. The present author is unable to accept VANBREUSEGHEM'S (2) interpretation of QUINCKE'S α-Pilz, namely that it was a species of *Microsporum*. VANBREUSEGHEM'S main reason for holding this view seems to have been based on QUINCKE'S drawing of the macroconidia of his α-Pilz which showed three with long-drawn out tips and one with a blunt tip; he also considered that the yellow pigment which was present on the reverse of QUINCKE'S α-Pilz cultures was additional support for his contention, since, although he himself had observed this pigment on the reverse of his own cultures, it was modified by the presence of a centrally situated patch of violet pigment. BODIN (l.c.) had also observed this violet pigment in his cultures but evidently did not consider that it had any taxonomic significance. The present author is also unable to accept BLANK'S contention that not only QUINCKE'S but his own isolates, were a species of *Microsporum*, viz. *M. quinckeanum* (ZOPF) GUIART & GRIGORAKI, although it is clear that his reasons for this were not the same as those put forward by GUIART & GRIGORAKI as may be observed on reading their definition of the genus *Microsporum* which was quoted in Part I of this work. While his description of the macroconidia observed by him obviously fits that which is generally applied to the macroconidia of the genus *Microsporum* (quite the opposite of that given by the present author for his strains of *T. quinckeanum*) his illustrations, figs. 3—6 incl. could represent some of the features, including the macroconidia, of any of the present author's isolates; even the macroconidia represented in his fig. 7, which incidentally do not appear to have thick walls, but which are spindle-shaped and pointed at their distal end, have been observed from time to time amongst the macroconidia produced by the present author's isolates. Likewise, BLANK'S illustrations of microconidia resemble those seen by the author, and by their occurrence in great numbers, as shown by BLANK in his fig. 6 are more characteristic of microconidia of *Tricho-*

phyton species and also, while it is true that in *Microsporum* species microconidia are pyriform to elongate, this is equally true of many *Trichophyton* species. Lastly, BLANK did not find any animal or human hairs invaded by this dermatophyte and therefore claimed that, in this inability to invade hair in the living host it had a feature in common with *Microsporum gypseum*; but KAPLAN, GEORG & FOSNAUGH (12) recorded and illustrated invasion of the hair in the case of a horse which was spontaneously infected by this dermatophyte.

In concluding this discussion the author wishes to summarise his views as follows:

Firstly, the authenticity of QUINCKE'S α-Pilz and the claims he made for it are upheld by the results of the author's own investigations; secondly, on the basis of its parasitic morphology and its saprophytic morphology in culture, he maintains that the dermatophyte which has been the subject of these investigations is a member of the genus *Trichophyton*; finally, on the basis of its microscopic morphology in culture, he considers that it sufficiently resembles the various dermatophytes included by CONANT et al. (14) in the group species *Trichophyton mentagrophytes* (ROBIN) BLANCHARD to justify its being included with them by these authors; but, nevertheless, he wishes to emphasize that it is clearly distinguishable from the other members of this group species in several other respects, namely: its ability to produce scutula in hair-bearing animals and occasionally in Man; its larger arthrospores in infected hair follicles; and lastly, its ability to cause, in Wood's light, a green fluorescence in animal hair invaded by it.

ACKNOWLEDGEMENT

The author gratefully acknowledges the gift of samples of culture media from Oxo Ltd., London. He also acknowledges the help of his technician Mr. R. A. FORSTER in the preparation of the culture media used in the foregoing experiments.

References

1. BODIN, E. 1902: Sur le champignon du favus de la souris. (*Achorion quinckeanum*). Arch. Parasitol. **5:** 5—20.
2. VANBREUSEGHEM, R. 1950: Position systématique et nomenclature de l'-*Achorion Quinckeanum*. Ann. Parasitol. **25:** 188—199.
3. BLANK, F. 1957: Favus of mice. Canad. J. Microbiol. **3:** 885—896.
4. RIDGWAY, R.: Color standards and color nomenclature. 1912; Washington, D.C.
5. RIVALIER, E. 1934: La culture des champignons des teignes sur lames gelosées. Ann. Dermatol. Syphil. 7e. ser. **5:** No. 8: 780—786.
6. LANGERON, M. & MILOŠEVIĆ, S. 1930: Morphologie des dermatophytes sur milieux naturels et milieux à base de polysaccharides. Essai de classification. Ann. Parasitol. **8:** 465—508.
7. QUINCKE, H. 1887: Ueber Favuspilze. Arch. exp. Pathol. Pharmakol. **1** and **2:** 62—76 (delivered in 1886).
8. MEWBORN, A. 1903: Report of a case of favus of scrotum coexisting with ringworm of the thigh giving identical Trichophyton -like cultures. J. Cut. Diseases; **21:** No. 244: 11—17.

9. Vanbreuseghem, R. & Borgers, G. 1951: A propos d'une souche de *Sabouraudites* (*Microsporum*) *gypseus* isolée au Congo belge. Ann. Soc. belge Med. trop. **31:** No. 3: 377—382.
10. Emmons, C. W. 1934: Dermatophytes. Natural grouping based on the form of the spores and accessory organs. Arch. Derm. & Syph. **30** No. 3: 337—362.
11. Sabouraud, R. 1910: Les Teignes. Masson & Cie. Paris.
12. Kaplan, W., Georg, L. K. & Fosnaugh, C. J. 1956: Isolation of the dermatophyte, *Microsporum gypseum,* from a horse with ringworm. J. A. V. M. A. **129,** No. 8: 381—383.
13. Forster, R. A. 1956: A useful method for the addition of antibiotic to culture media. J. Med. Lab. Tech. **13:** 417—418.
14. Conant, N. F., Smith, D. T., Baker, R. D., Callaway, J. I. & Martin, D. S.: Manual of clinical mycology. 2nd. ed. 336, 1954. W. B. Saunders Co. Philadelphia.

Addenda

Part 1 (in vol. XI)

References

37. Mewborn, A. 1903: Report of a case of favus of scrotum coexisting with ringworm of the thigh giving identical Trichophyton-like cultures. J. Cut. Diseases. **21,** No. 244: 11—17.
38. Blank, F. 1957: Favus of Mice. Canad. J. Microbiol. **3,** 885—896.

Errata

Part 1 (in Vol. XI)

Read magnifications as follows:

Page 265. Plate II. Fig. 1. approx. 3/4 nat. size. Fig. 2. approx. nat. size.
Page 272. Plate III. Fig. 1. approx. 1 ½ times nat. size. Fig. 2. approx. 1 ½ times nat. size.
Page 263. Table I. read across: J. H. male. 14 yr. Strain No. 2 scalp 1, face 1.

N.B. Dr. G. C. Ainsworth (personal communication) has pointed out that MacLeod & Muende (24) mention the binomial *T. quinckeanum* on p. 361.

LA MICOLOGÍA MÉDICA EN MÉXICO
REVISIÓN DE LA BIBLIOGRAFÍA APARECIDA DURANTE EL PERÍODO DE 1946 A 1958.

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(13.X.1959)

Recientemente uno de nosotros (GONZÁLEZ OCHOA, 1955a) presentó una comunicación sobre el status de las enfermedades por hongos en México, en la que se ponía de manifiesto que, dadas las variadas condiciones biogeográficas del país, todas las enfermedades por hongos descritas en la bibliografía cosmopolita habían sido observadas en México, con excepción de la piedra negra o tropical.

Aunque la presente revisión comprende las aportaciones micológicas publicadas en el período de 1946 a 1958, se han hecho unas cuantas excepciones cronológicas en ciertos casos que consideramos dignos de mención dado el significado que han tenido en la difusión de la especialidad en el país. Las publicaciones a que haremos referencia, durante el período 1946—1958, son las que consideramos de interés y que han aportado algún dato original al conocimiento de la micología mexicana, haciendo caso omiso de multitud de comunicaciones sobre casos clínicos en que sólo se citan otras observaciones más de padecimientos micológicos comunes en nuestro medio. En la presente exposición seguiremos el ordenamiento con el que se han venido presentando esta clase de revisiones micológicas de los diversos países aparecidas en *Mycopathologia et Mycologia Applicata*, o sea el de agrupar los datos de experimentación, de clínica o de terapéutica micológica en concordancia con las diversas micosis, incluyendo un párrafo sobre micología general y, además, un mapa con la situación geográfica y división política de la República Mexicana, ya que en ocasiones se hacen referencias a ciertas entidades del país, donde han sido observadas especialmente algunas micosis.

DERMATOMICOSIS

Las dermatomicosis son, como en cualquier otro país, las enfermedades por hongos más frecuentes, particularmente las dermatofitosis. Para facilitar la revisión de este grupo de micosis superficiales se mencionarán separadamente las dermatofitosis, la pitiriasis versicolor, el eritrasma, la tinea nigra y la moniliasis superficial.

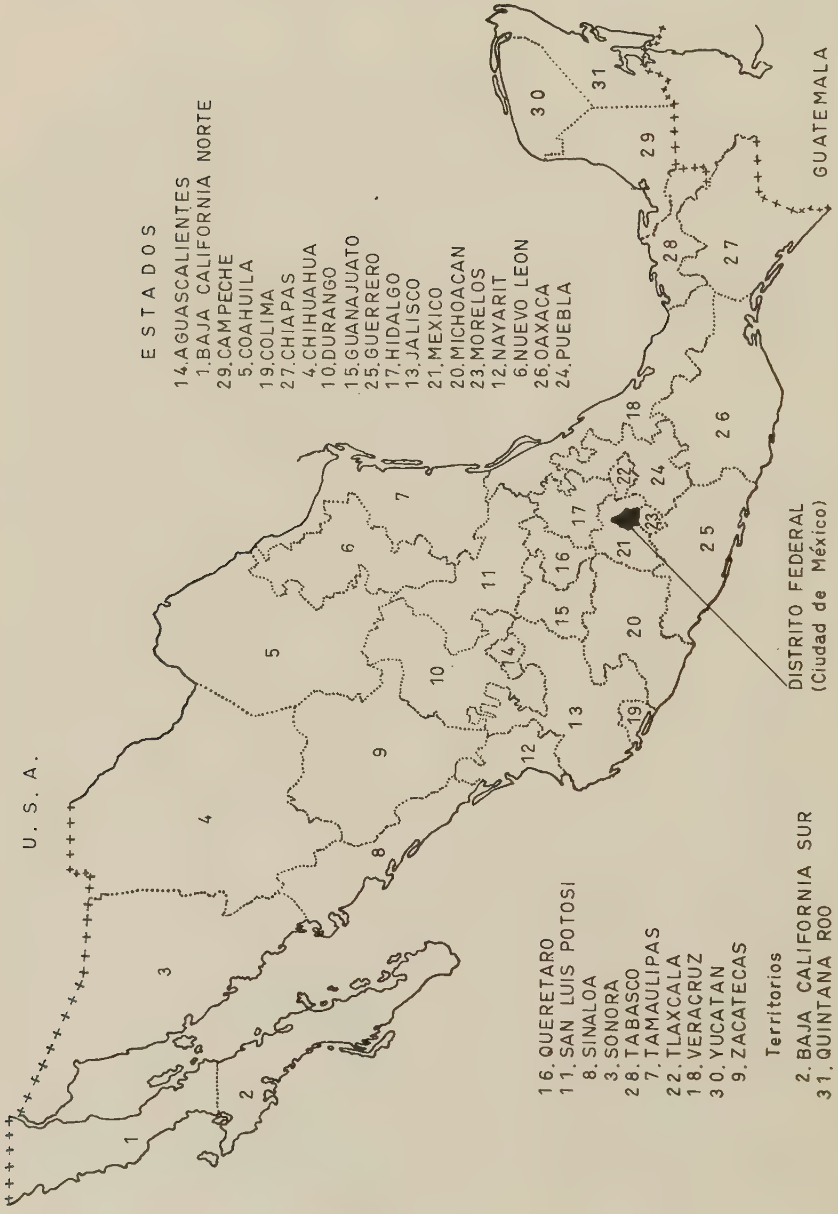
Dermatofitosis.

Prácticamente todas las especies de dermatofitos, así como las formas clínicas y localizaciones de las lesiones que producen, han sido observadas en México, pero existe un hecho llamativo por lo que se refiere a la frecuencia de *Trichophyton tonsurans*, la que sin duda es la especie de mayor importancia en México, no sólo por lo que toca a la tinea capitis de la que prácticamente el 90% es debida a esta especie, sino por lo que se refiere también a la producción de lesiones ungueales y de la piel lampiña.

a) Tinea capitis. — GONZÁLEZ OCHOA & ROMO (1945) realizaron uno de los primeros estudios tendiente a investigar las diversas especies de dermatofitos causantes de la tinea capitis en nuestro medio; para este efecto fueron estudiados 268 casos, en los que se pudo observar que las tiñas del tipo tricofítico (91.8%) predominaron sobre las microspóricas (8.2%). De las especies correspondientes al género *Trichophyton*, *T. tonsurans* alcanzó 89.4% de las cepas aisladas, y el resto correspondieron a *T. mentagrophytes*, *T. Schönleini* y *T. violaceum*. En las tiñas microspóricas la única especie aislada fué *Microsporum canis*.

Tal vez la contribución mexicana de mayor importancia a la micología médica es el tratamiento de la tinea capitis por la depilación con el acetato de talio, por lo menos hasta antes del advenimiento de la griseofulvina. A CICERO (1918) se debe el haber puntualizado la dosis depilatoria carente de efectos tóxicos de importancia, y demás normas del tratamiento. Posteriormente otros dermatólogos mexicanos entre los que se debe mencionar a GONZALEZ HERREJÓN (1919) y a GONZÁLEZ URUEÑA (1928) participaron en la difusión de este recurso terapéutico que llegó a emplearse en otros paises como Inglaterra, Italia, España, Rusia y Marruecos Español (BRUMPT, 1949). Durante el período que cubre la revisión bibliográfica presente aparecieron varias comunicaciones, entre las que se encuentran las de HAYASHI BECERRA (1952), BARBA RUBIO & PEREZ SUÁREZ (1956) y ACEVES ORTEGA (1956a); en las que se anotan los resultados satisfactorios obtenidos y se insiste sobre la bondad del tratamiento.

b). — Tinea pedis, corporis, cruris, favosa e imbricata. — GONZÁLEZ OCHOA & LAVALLE (1947) estudian la frecuencia de los diversos hongos causantes de las tiñas de la piel lampiña, utilizando el material recogido en 52 casos estudiados. Como resultado de esta exploración se obtuvieron los siguientes datos: *T. mentagrophytes* fué aislado en el 30.9% de los casos, le siguieron en orden de frecuencia *M. canis*, *T. rubrum*, *T. tonsurans*, *E. floccosum* y


U. S. A.
E S T A D O S
14. AGUASCALIENTES
1. BAJA CALIFORNIA NORTE
29. CAMPECHE
5. COAHUILA
19. COLIMA
27. CHIAPAS
4. CHIHUAHUA
10. DURANGO
15. GUANAJUATO
25. GUERRERO
17. HIDALGO
13. JALISCO
21. MEXICO
20. MICHOACAN
23. MORELOS
12. NAYARIT
6. NUEVO LEON
26. OAXACA
24. PUEBLA
16. QUERETARO
11. SAN LUIS POTOSI
8. SINALOA
3. SONORA
28. TABASCO
7. TAMAULIPAS
22. TLAXCALA
18. VERACRUZ
30. YUCATAN
9. ZACATECAS
Territorios
2. BAJA CALIFORNIA SUR
31. QUINTANA ROO
DISTRITO FEDERAL
(Ciudad de México)
GUATEMALA

T. concentricum. En este estudio se señalan además algunos aspectos clínicos, epidemiológicos e inmunológicos de ciertas tiñas de la piel lampiña.

Después de la primera mención sobre la existencia de la tinea imbricata en el país (González Ochoa & Lavalle, 1947), Núñez Andrade (1953) comunica el hallazgo de siete casos y presenta datos epidemiológicos, clínicos, de laboratorio, de anatomía patológica y de tratamiento sobre esta dermatofitosis, y Estrada & Lavalle (1956) señalan el primer caso autóctono de la Ciudad de México.

c.) — Tinea ungium. — En fecha más reciente (González Ochoa & Orozco 1957) se han completado los estudios sobre la repartición de las especies de dermatofitos en las diferentes localizaciones de las dermatofitosis en México, encontrándose que en la tiña de las uñas se aisla con mayor frecuencia *T. rubrum*, seguido de *T. tonsurans* y *T. mentagrophytes*.

Otros aspectos en relación con las dermatofitosis. — La primera y única comunicación que indica la exisrencia de *Microsporum gypseum* en México es la Schnaas & González Ochoa (1958) quienes señalan su existencia en la tiña del perro.

González Ochoa & Córdova (1957) realizan un estudio tendiente a demostrar la importancia del factor sensibilización en las dermatofitosis de la piel lampiña, para este efecto se llevaron a cabo inoculaciones con dermatofitos (*T. mentagrophytes*, *T. concentricum* y *M. canis*) en la cara interna del antebrazo de voluntarios normales, las que produjeron una placa eritematoescamosa con vesiculación macrosópica en determinado número de sujetos a partir del cuarto día, y cuyo estudio histopatológico mostró espongiosis, edema intracelular y formación de vesículas; los mismos inoculi, aplicados en las mismas condiciones, en el otro antebrazo del mismo sujeto, pero adicionados de fluorocortisona a la concentración de 0.2%, evitó la respuesta inflamatoria en todos los sujetos; la aplicación de los mismos inoculi, en las mismas condiciones, pero sometidos al autoclave (20 minutos a 15 lbs) produjeron el mismo tipo de respuesta inflamatoria que en el caso primero en los sujetos previamente sensibilizados a los dermatofitos, solo que a los 8 ó 10 días de incubación; y estos mismos inóculos muertos, adicionados de fluorocortisona, no produjeron la respuesta eczematosa. De lo anterior concluyeron que el mecanismo fundamental en las dermatofitosis de la piel lampiña, es el de sensibilización, produciéndose una lesión alérgica eczematosa.

Revisiones en las que se analizan algunos aspectos clínicos, de diagnóstico, frecuencia y tratamiento de las dermatomicosis han sido publicadas por Germes Leal (1953) y González Ochoa (1955b)

Pitiriasis versicolor.

Esta dermatomicosis es sumamente común en las zonas costeras del país, tanto del Golfo de México como del Océano Pacífico.

González Ochoa (1955a) señala la predominancia de las formas acrómicas que deben ser consideradas en el diagnóstico diferencial del mal del pinto, particularmente en el Estado de Chiapas donde ambos padecimientos existen y la pitiriasis versicolor presenta localizaciones poco usuales, como en las extremidades. El mismo autor (González Ochoa 1956b) revisa las diferentes formas clínicas del padecimiento y hace consideraciones sobre la distribución geográfica, diagnóstico diferencial, diagnóstico micológico y tratamiento; por lo que se refiere al diagnóstico micológico llama la atención sobre la posible confusión de *Malazessia furfur* con *Pityrosporum ovale.*

Eritrasma.

Con relación a esta micosis superficial, de repartición cosmopolita, González Ochoa (1955a) señala que en algunas zonas tropicales del país existen casos de localizaciones aberrantes como son las de la región inferior del abdomen, espacios interdigitales del pie y otras partes del cuerpo donde las superficies cutáneas permanecen en contacto, como el surco interglúteo y los pliegues inframamarios.

Tinea Nigra.

Sobre esta dermatomicosis no existen más aportaciones que la de González Ochoa (1955a) señalando su existencia en el país y su rareza.

CANDIDIASIS O MONILIASIS

Comprendemos bajo el término moniliasis, que en México se sigue prefiriendo al de candidiasis, a las infecciones originadas por levaduras del género *Candida*, particularmente por *C. albicans*, estableciéndose el distingo entre moniliasis superficial y moniliasis profunda o metastática, considerando bajo el término de moniliasis superficial a las infecciones que producen esas levaduras en la piel, las uñas y mucosas externas, infecciones que por su patogenia, sintomatología y benignidad, son claramente distintas de las moniliasis profunda o metastática.

Las contribuciones mexicanas acerca ce este tema se presentarán bajo tres aspectos: estudios sobre levaduras del género *Cándida*, estudios en relación con la moniliasis superficial y estudios en relación con la moniliasis metastática.

a) Levaduras del género Cándida.

González Ochoa & Sandoval (1956a) presentan una exploración acerca de la frecuencia y repartición de levaduras del género *Candida* en materias fecales diarreicas, antes de la difusión de los antibióticos de amplio espectro, o sea datos obtenidos en 1947; con datos similares obtenidos en plena época de generalización de esos antibióticos; sus resultados muestran que de un 44% de portadores de *C. albicans* encontrado en 1947, las cifras aumentaron a un 70%

en 1956, y por lo que se refiere a la repartición de las especies del género *Candida* parece que dichos antibióticos han influído en el incremento de *C. tropicalis* y la disminución de *C. pseudotropicalis*.

Posteriormente GONZÁLEZ OCHOA (1957b), dando por aceptado el incremento de levaduras del género *Candida*, como consecuencia de la terapia por antibióticos de amplio espectro y sulfonamidas, insiste enfáticamente en que no debe ser confundido el aumento en el número de portadores asintomáticos de grandes cantidades de levaduras, lo que es un hecho biológico, con el aumento en la frecuencia de moniliasis, o sea el hecho patológico, el cual ha sido exagerado; en el mismo trabajo el autor puntualiza algunas precauciones que deben tomarse en cuenta antes de establecer un diagnóstico micológico de moniliasis. DURAZO (1956) publica una nota en relación con el diagnóstico de laboratorio de la moniliasis.

GONZÁLEZ-MENDOZA & BOJALIL (1958) efectúan un estudio tendiente a conocer la frecuencia de *C. albicans* en el esputo de enfermos tuberculosos; comparan sus resultados con los obtenidos por otros autores en estudios similares realizados antes de la introducción de la estreptomicina en el tratamiento de la tuberculosis, y deducen que dicho tratamiento no ha modificado la frecuencia de la levadura en el esputo de los enfermos tratados con ese antibiótico.

SÁNCHEZ MARROQUIN (1958) estudia con fines taxonómicos algunas relaciones existentes entre diversas especies del género *Candida*, teniendo como base caracteres morfológicos, fisiológicos y bioquímicos; encuentra que *C. lipolytica*, *C. zeylanoides*, *C. tropicalis*. *C. robusta*, *C. curvata*, *C. solani*, *C. utilis* y *C. humicola* son especies bien definidas, y que *C. guillermondi* y *C. melibiosi* son una misma especie, lo mismo que *C. albicans* y *C. clausenii*; por otra parte *C. parapsilosis* y *C. guillermondi* var. *membranaefaciens* están estrechamente relacionadas aunque presentan algunas diferencias bioquímicas. El autor concluye que cuando se emplea un número reducido de compuestos de carbono frecuentemente se observan similitudes que conducen a apreciaciones falsas, por lo que es de recomendarse la utilización sistemática de un número más grande de los mencionados compuestos.

b) Moniliasis superficial.

GONZÁLEZ OCHOA *et al.* (1955) ensayan la nystatina en 75 casos de moniliasis oral del niño; sus resultados muestran que el antibiótico fué de un valor terapéutico excelente. En fecha posterior GONZÁLEZ OCHOA & DOMINGUEZ (1957a) realizan un trabajo tendiente a esclarecer algunos puntos en relación con la patogenia y epidemiología de la moniliasis oral del recién nacido; para el efecto fueron estudiados los casos en tres Instituciones de diverso nivel económico y social en la Ciudad de México, en las que se investigaron los siguientes datos: a) frecuencia de la moniliasis en niños a término y en prematuros, b) frecuencia de la enfermedad en relación con la permanencia del niño con la madre o separado en cuna, c) frecuencia del padecimiento en relación con la alimentación al pecho o con biberón, d) existencia de contactos moniliásicos, principalmente la madre, y e) aplicación previa de antibióticos en los niños y en las madres. De sus resultados concluyen que las circumstancias que parecen condicionar de manera más directa el padecimeinto, exceptuando el nacimiento prematuro, parecen ser el contacto íntimo del niño (en la misma cama) con la madre y la alimentación al pecho.

SALAZAR MALLÉN (1957) discute la importancia de los antígenos de *Candida* y *Monilia* en relación con algunos problemas de índole clínico y terapéutico en la alergia.

c) Moniliasis profunda o metastática.

QUIJANO (1958) discute algunos aspectos de la moniliasis como posible complicación post-operatoria en las intervenciones quirúrgicas y la relación que dicha situatión puede guardar con la administración de antibióticos.

MICETOMA

Dentro del síndrome micetoma, compuesto por tumoración, fístulas y granos, el micetoma actinomicósico es mucho más importante que el maduromicósico, éste último representa menos aún que el 1% de los casos de micetoma observados en México.

Micetoma Actinomicósico.

El micetoma por *Nocardia brasiliensis*, juntamente con la esporotricosis, constituyen uno de los principales problemas de la patología micológica nacional. En los últimos 10 años cabe hacer mención de diversos trabajos en los que se comunican casos clínicos con alguna peculiaridad, problemas de tratamiento o se hacen revisiones del tema. Entre estas publicaciones se encuentran la de LAVALLE & MILLÁN (1949) quienes describen un caso extraordinariamente crónico de micetoma de la mano producido por *Nocardia pelletieri*. GARCÍA (1950) publica un caso de micetoma por *N. brasiliensis* localizado a la extremidad superior izquierda, el que reviste interés por ser uno de los primeros en que se menciona el éxito del tratamiento con diamino-difenil-sulfona. GONZÁLEZ OCHOA (1953a) discute la similitud clínica del micetoma de torax con invasión pulmonar por *N. brasiliensis* y la actinomicosis pulmonar con invasión de las paredes del torax, comprediendo este cuadro clínico con el término de micetoma tóraco-pulmonar, señalando algunos aspectos patogénicos para establecer el distingo entre ambas etiologías e insiste en la necesidad de puntualizarlas dada la diversidad de tratamiento. ROMO DIEZ (1955) comenta también algunos aspectos sobre la patogenia y formas clínicas de la actinomicosis con relación al micetoma y discute algunos problemas relacionados con el tratamiento de estas entidades clínicas. ACEVES (1958) presenta 68 casos observados en la ciudad de Guadalajara, Estado de Jalisco, en los que además de comentar sus aspectos clínicos y lo referente a tratamiento, menciona la distribución por sexos, edad, ocupación y localización geográfica de sus casos. LATAPÍ (1956) publica un amplio estudio del micetoma actinomicósico que incluye desde el aspecto histórico de la enfermedad en nuestro medio hasta los más recientes problemas relacionados con el tratamiento, presentando el estudio y análisis de 100 casos.

Diversos trabajos se han llevado a cabo sobre la sensibilidad de los actinomycetes causantes de micetoma a diversos fármacos. BOJALIL & SHIELS (1950) estudian la actividad *in vitro* de la diazona, pronamida, furacín y ácido p-amino salicílico sobre *N. brasiliensis*, *N. asteroides*, *N. madurae* y *N. pelletieri*; sus resultados señalan que las sulfonas estudiadas inhiben el crecimiento de *N. brasiliensis* a concentración de 0.02 mg/ml. GONZÁLEZ OCHOA *et al.* (1952) exploran la acción de la 4.4 diamino-difenil-sulfona (DDS) sobre *N. brasiliensis*, tanto *in vitro* como en la infección experimental del ratón y en casos humanos de micetoma; los resultados obtenidos ponen de manifiesto la sensibilidad del microorganismo *in vitro* y la efectividad del tratamiento en los casos humanos, pero en la infección experimental no obtuvieron resultados concluyentes. GONZÁLEZ OCHOA & MACOTELA (1955) notifican la inutilidad del vainillato de etilo y de la pregnenolona, en el tratamiento del micetoma actinomicósico por *N. brasileinsis*, medicamentos considerados, en aquella época, como de utilidad en el micetoma y la nocardiasis. Posteriormente GONZÁLEZ OCHOA & AHUMADA (1958) comunican las ventajas de la forma inyectable de la DDS, sobre la vía oral, en ciertos casos de micetoma por *N. brasiliensis*, particularmente la de una reducción notable en la duración del tratamiento.

En el capítulo de estudios inmunológicos de los actinomycetes, GONZÁLEZ OCHOA & VÁZQUEZ (1953) presentan un estudio sobre las relaciones serológicas de las principales especies patógenas, puestas de manifiesto por reacciones de precipitación; en su estudio incluyen 21 cepas, algunas de la misma especie, otras con diferentes nombres pero consideradas por los autores como sinonimias, especies válidas y *Streptomyces* no patógenos; sus resultados muestran que *N. asteroides*, *N. brasiliensis* y *A. bovis* tienen un antígeno común entre sí, pero que difiere de los de *N. madurae*, *N. pelletieri*, *N. somaliensis* y *N. paraguayensis*, así como de los *Streptomyces* no patógenos incluídos en el estudio; de las anteriores especies *N. madurae* y *N. pelletieri* muestran precipitación cruzada; *N. somaliensis* solo precipita en presencia del suero homólogo, mientras que *N. paraguayensis* cruza con *Str. albus*, *Str. griseus* y *Str. lavendulae*. GONZÁLEZ OCHOA & BARANDA (1953) trabajan sobre una intradermorreacción en casos de micetoma por *N. brasiliensis*, con un carbohidrato extraído de ese actinomiceto y encuentran que dicha reacción tiene especificidad suficiente como para ser considerada de valor diagnóstico, pero observan resultados negativos en casos de micetoma muy avanzados y con gran ataque al estado general, relacionando esa anergia con un mal pronóstico. El estudio de González Ochoa & Vázquez (1953), sobre las relaciones serológicas de algunos actinomycetes, ya mencionado, conduce a González Ochoa & Sandoval (1955) a revisar la posición taxonómica de las especies *madurae*, *pelletieri* y *paraquayensis* que venían siendo consideradas como pertenecientes al género *Nocardia*; estos autores sugieren que dichas especies sean trasladadas al género *Streptomyces* en vista de que no fragmentan y producen conidios en medios naturales. Posteriormente estos mismos autores (González Ochoa & Sandoval 1956b) hacen una revisión de las supuestas especies de actinomycetes causanes de micetoma descritas en la literatura y tomando en cuenta las especies que se aislan en México, país donde el micetoma actinomicósico es un padecimiento muy frecuente, concluyen que la especie más importante es *N. brasiliensis*, en mucho menor proporción *Str. madurae*, *Str. pelletieri* y excepcionalmente *N. asteroides*; además, presentan un estudio morfológico y bioquímico de las especies descritas como *N. leishmanii*, *N. convoluta* y *N. phenotolerans* sugiriendo que deben caer en sinonimia de *N. asteroides*, mientras que las especies *N. transvalensis* y *N. pretoriana* serían sinonimias de *N. brasiliensis*.

Micetoma Maduromicósico

Este tipo de micetoma, como ya se señaló, es muy raro en México. González Ochoa & Orozco (1953) han reportado casos causados por *Cephalosporium* y *Monosporium*, y Mendiola (1947) un caso producido por *Monosporium apiospermum* [1]).

NOCARDIASIS

Queremos pronunciarnos, una vez más, sobre la confusión lamentable que se hace entre los términos micetoma actinomicósico y nocardiasis. González Ochoa (1955a, 1957c) ha venido insistiendo sobre este error tan difundido, inclusive en textos especializados. El micetoma actinomicósico está constituído por tumoraciones fistulosas con granos formados por el apelotonamiento del micelio de un actinomycete, sea del género *Nocardia*, sea del género *Streptomyces*, mientras que el término nocardiasis (seudotuberculosis) connota infecciones granulomatosas pulmonares, meningo-encefálicas o subcutáneas, en las que el actinomycete causante, *Nocardia asteroides*, adopta el aspecto de cortos filamentos ramificados o formas bacilares ácido-resistentes, muy semejantes a *Mycobacterium tuberculosis*.

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[1]) Se ha conservado el nombre de *Monosporium apiospermum* en lugar de *Allescheria boydii*, por ser aquel el que aparece en las publicaciones mencionadas.

En México han sido observados casos de nocardiasis de localización pulmonar, meningo-encefálica y subcutánea (González Ochoa, casos no publicados) de los que ha sido aislada *N. asteroides*.

ACTINOMICOSIS

La relativa frecuencia con que se observan infecciones por *Actinomyces bovis* en México, preferentemente en su localización cérvico-facial, explica la carencia de comunicaciones por considerárseles como comunes. Desde el punto de vista clínico, a la actinomicosis cabría considerarla como un micetoma actinomicósico debido a un actinomycete anaerobio (González Ochoa 1957c), puesto que se trata de tumoraciones fistulosas con granos actinomicósicos; pero la diversa distribución geográfica de la actinomicosis (cosmopolita) y del micetoma (regiones tropicales y subtropicales) así como la diversidad en la patogenia de la infección, localización de las lesiones y habitat de los microorganismos causantes, son factores que explican la reticencia de los autores de países donde no existe el micetoma para considerar a la actinomicosis como un micetoma.

Algunos trabajos en relación con *A. bovis* y la infección que produce ya fueron citados a propósito del párrafo correspondiente a micetoma actinomicósico, como son los relativos a la localización tóraco-pulmonar de la actinomicosis (González Ochoa 1953a) y las relaciones serológicas que *A. bovis* tiene con nocardias (González Ochoa & Vázquez, 1953). González Ochoa & Sandoval (1949) presentaron los resultados de una exploración sobre la susceptibilidad de 3 cepas de *A. bovis, in vitro,* a la sulfadiazina, penicilina, estreptomicina y promina, encontrando que la penicilina tiene mayor actividad y constancia, a ésta le sigue la sulfadiazina; que la estreptomicina es de acción más variable, lo que parece condicionado a la cepa y finalmente que la promina solamente produce ligera inhibición a concentraciones altas.

ESPOROTRICOSIS

Dada la frecuencia de la esporotricosis en el país, tal vez sea en este padecimiento donde México ha contribuído en el conocimiento de algunos aspectos de esta micosis. Los conceptos fundamentales del tema se encuentran resumidos por González Ochoa en el capítulo sobre esporotricosis del "Handbook of Tropical Dermatology" editado por Simons (1952); en esta se presenta una clasificación patogénica de la esporotricosis, la que ha sido adoptada ya por otros autores, y que tiene como base el futuro que siga *Sporotrichum schenckii*, después de su entrada al tegumento, es decir, a partir del chancro esporotricósico, sea que se disemine por vía linfática, sea que quede *in situ,* o que se disemine por vía sanguínea; estas situaciones condicionarían tres tipos, el tipo linfangítico, el fijo y el hematógeno respectivamente. Estos tipos comprenden formas clínicas que tienen como base aspectos morfológicos. En el tipo linfangítico habria dos formas clínicas: gomosa ascendente de las extremidades, y gomosa de otras regiones; la razón para establecer estas dos formas clínicas se debe a la sistematización morfológica de los linfáticos en las extremidades, a diferencia de los de cualquiera otra región, lo que conduce a la tan característica imagen clínica de lesiones gomosas de aparición ascendente, siguiendo lîneas más o menos paralelas, cuando la esporotricosis de diseminación linfática asienta en las extremidades, y que difiere de las gomas o nódulos, sin ordenamiento alguno, cuando aparecen en cara, cuello o tronco. El tipo fijo, en el que no hay diseminación, sólo se produciría un agrandamiento del chancro, el que adopta aspectos ulceroso, verrucoso, acneiforme, de placa infiltrada, o de placa eritemato-escamosa (psoriasiforme), que caracterizan esas cinco formas clínicas. En el tipo hematógeno la diseminación sanguínea de *S. schenckii* produce la aparición de los elementos gomosos en forma bilateral, sobre todo el tegumento, de lo que resulta la forma clínica gomosa diseminada. En ese mismo capítulo se menciona que a medida que se ha ensayado la reacción cutánea con el polisacárido de *S. schenckii* (GONZÁLEZ OCHOA & SOTO FIGUEROA, 1947) se ha comprobado, cada vez, su alta especificidad. Otros artículos de revisión general del tema son el de ACEVES (1950) y el publicado por LAVALLE (1956a), en este último se menciona un caso con participación ósea.

En el órden de los conocimientos clínicos SÁNCHEZ MARROQUIN (1947) menciona algunas de las características clínicas de casos observados y expone algunos datos acerca de la morfología microscópica y el cultivo de *S. schenckii*. LATAPÍ (1950), en una nota sobre esporotricosis facial infantil, discute la frecuencia de esa localización y presenta un caso al respecto.

En relación con los aspectos inmunológicos el trabajo de GONZÁLEZ OCHOA & SOTO FIGUEROA (1947), a que se ha hecho mención, se refiere a la obtención de dos polisacáridos obtenidos de S. *schenckii*, los que no se muestran antigénicos, pero son capaces de precipitar con los sueros homólogo y heterólogo de conejo y con el suero de casos humanos de esporotricosis, si bien este hecho no es constante, en cambio inyectados intracutáneamente producen una reacción inflamatoria en los casos de esporotricosis. GONZÁLEZ OCHOA & SALINAS (1953) vuelven a tomar el estudio de los polisacáridos de *S. schenckii* y encuentran que las reacciones de precipitación muestran resultados inconstantes y que las de fijación del complemento no pueden ser utilizadas porque los polisacáridos se muestran anticomplementarios, pero confirman la especificidad de ese antígeno en las pruebas cutáneas utilizadas en el diagnóstico, observando que la reactividad cutánea tiende a desaparecer al curar la enfermedad.

Por lo que se refiere a estudios micológicos de *S. schenckii* GONZÁLEZ OCHOA & SOTO PACHECO (1950) estudian el desarrollo del hongo a partir de pus tomado de gomas esporotricósicas colocado únicamente en presencia de suero fisiológico y penicilina; señalan los autores el polimorfismo del aspecto parasitario del hongo encontrado dentro de las células del pus y a partir de estas formaciones describen el ciclo del hongo desde la fase parasitaria hasta la producción de la fase micelial. Como consecuencia de la observación anterior, GONZÁLEZ OCHOA *et al* (1950) notifican el efecto estimulante que ejerce la penicilina sobre el desarrollo de *S. schenckii* y las consecuencias que esto puede tener si se emplea dicho antibiótico en el tratamiento de la esporotricosis.

Finalmente, GONZÁLEZ BENAVIDES (1952) tomando en cuenta la significación que tiene el zacate de empaque en la trasmisión de la esporotricosis, observada por varios investigadores mexicanos, y la frecuencia con que el padecimiento se presenta en los alfareros, sugiere se le considere como enfermedad profesional entre los que se dedican a esa actividad, basando esta sugestión en la observación de 16 casos.

CROMOMICOSIS

El primer caso mexicano fué diagnosticado histológicamente por MARTÍNEZ BÁEZ (1940) y confirmado con el aislamiento de *Hormodendrum pedrosoi* por GONZÁLEZ OCHOA (1941). A partir de esa fecha la enfermedad ha sido observada con relativa frecuencia en sujetos procedentes de diversos estados de la República; sin embargo, los numerosos casos encontrados permanecen inéditos o son presentados en sesiones clínicas, y solo excepcionalmente se han hecho publicaciones al respecto; un ejemplo de estas es la de ACEVES (1956b) en que presenta algunos casos de cromomicosis juntamente con otros padecimientos producidos por hongos. RODRÍGUEZ (1956) presenta una revisión del tema y comunica dos casos tratados con calciferol, de los que uno curó clínica e histológicamente. Más recientemente, NOVALES (1958) comunica un caso tratado satisfactoriamente con diamino-difenil-sulfona; el empleo de este fármaco en algunos casos de cromomicosis ya había sido señalado por GONZÁLEZ OCHOA (1955a). GUERRERO SANTOS (1956) relata los buenos resultados de la extirpación quirúrgica de la piel invadida, inclusive en casos extensos de cromomicosis, seguida de la consiguiente aplicación de injertos; fundamenta este recurso en tres casos tratados con éxito.

COCCIDIOIDOMICOSIS

La primera comunicación sobre un caso autóctono de coccidioidomicosis fué la presentada por MADRID (1948) quien observó el padecimiento en un individuo originario de Sonora (Estado fronterizo del noroeste del país con los Estados Unidos de Norteamérica) sin embargo, el mismo autor hace la aclaración de que su enfermo había residido durante 6 meses en Arizona, U.S.A., 16 años antes de las manifestaciones sintomáticas; en esa misma comunicación el autor expone los resultados de una encuesta sobre reactividad cutánea a la coccidioidina en la población de Hermosillo, Estado de Sonora, practicada en 213 personas, la que acusó 22 % de positividad en hombres y 8.2 % en mujeres. En una segunda publicación el mismo MADRID (1950) notifica la existencia de tres casos de coccidioidomicosis en sujetos que nunca habían salido de Sonora, y presenta algunas consideraciones sobre el tratamiento quirúrgico de esta micosis.

Posteriormente se han observado multitud de casos, no solo de los Estados fronterizos del norte de México, sino de otras regiones alejadas de esa zona; así PÉREZ REYES & LARRÉ (1951) y LAVALLE (1956b) comunican dos casos en el Estado de Michoacán, zona alejada de la frontera con los Estados Unidos de Norteamérica, donde se localiza el área de mayor endemicidad. DE LA GARZA & RODRÍGUEZ (1952) presentan el estudio micológico e histopatológico de cuatro casos de coccidioidomicosis observados en la Ciudad de Monterrey, Estado de Nuevo León; desde esa comunicación, a la fecha, estos autores han observado más de 50 casos en el Estado de Nuevo León [1]). Es de mencionarse el caso publicado por CARDONA & VELÁZQUEZ (1954) por tratarse de una localización casi exclusiva al sistema nervioso central.

Con objeto de conocer la frecuencia de personas que reaccionan en forma positiva a la coccidioidina, se han llevado a cabo encuestas epidemiológicas en diversas zonas del país: GONZÁLEZ OCHOA (1949) en la región noroeste del país señala una frecuencia de 17.2 %, la que se reduce al 13.4 % al descartar a los sujetos que habían estado en la zona endémica de los Estados Unidos de Norteamérica. OCHOA MARTÍNEZ & SANTOSCOY (1951) encuentran cifras de 13.7 % de positividad en el Estado de Jalisco. En la Ciudad de León, Estado de Guanajuato, LÓPEZ SANABRIA (1952) encuentra 21.5% de personas que reaccionan en forma positiva. Por último, SLIM VILLEGAS & ARANDA (1953) realizan la misma encuesta en 6704 personas residentes en el Valle de Mexicali, Estado de Baja California Norte, lugar fronterizo con el Estado de California, EE..UU., y encuentran una frecuencia de positividad del 20.1 %.

De los estudios anteriores, incompletos aun, se ha podido inferir que la zona norte, particularmente la zona noroeste de la República Mexicana constituye una área endémica extensa de coccidioidomicosis; sin embargo, aun son necesarios estudios epidemiológicos más amplios y precisos para poder delimitar dicha área.

Existen algunas publicaciones que se ocupan de la revisión general de la coccidioidomicosis, en las que se mencionan algunos aspectos del problema en México, como las de GONZÁLEZ OCHOA (1949, 1954) y LAVALLE (1956b).

Apoyados en la información de MOORE (1941) GONZÁLEZ OCHOA & MARCOR (1952) trataron de obtener la fase esferular de *Coccidioi-*

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[1]) Comunicación personal.

des immitis por su inoculación en el saco vitelino y la membrana corioalantoidea del embrión de pollo; para el efecto se inocularon dos series de huevos embrionados, una con la fase filamentosa y otra con esférulas obtenidas de testículo de cobayo infectado; en ambas situaciones los autores obtuvieron únicamente crecimiento micelial.

Por lo que se refiere a tratamiento de la coccidioidomicosis GONZÁLEZ OCHOA *et al* (1953) probaron el vainillato de etilo sin obtener beneficio con ese fármaco. GONZÁLEZ OCHOA & ROSILES (1953) reportaron la curación de un caso de coccidioidomicosis con 500 mgs diarios de hidroxicloroquina durante 60 días, sin que se observaron efectos colaterales indeseables. Este caso 6 años después continúa enteramente sano y se ha obtenido la curación en dos casos más (Datos no publicados de GONZÁLEZ OCHOA).

Finalmente, SCHNAAS *et al* (1957) señalaron un caso de infección natural por *C. immitis* en un perro procedente del Estado de Sinaloa.

HISTOPLASMOSIS

El primer caso de histoplasmosis en México fué comunicado por PERRÍN & MARTÍNEZ BÁEZ (1943), caso que tuvo la importancia en aquella época de ser diagnosticado meses antes de morir; desde entonces se han venido observando casos aislados de histoplasmosis enfermedad o diseminada, los que no han sido publicados, con excepción del caso de MENDIOLA & LICONA (1953); pero en tratándose de la infección primaria se observan con frecuencia epidemias de histoplasmosis pulmonar aguda primaria, epidemias que tienen lugar en espacios confinados, como cavernas o minas, que están constantemente asociadas con guano de murciélago, que se caracterizan por su gravedad, las que en ocasiones alcanzan mortalidad hasta del 40%, y finalmente que se han presentado en una zona geográfica muy amplia, ya que han sido observadas en los Estados de Coahuila, Durango, Tamaulipas, San Luis Potosí, Querétaro, Guerrero, Nayarit y Yucatán. Las características anteriores han sido analizadas por GONZÁLEZ OCHOA (1957a) quien presentó un estudio sobre 14 epidemias acaecidas en los 8 Estados mencionados, estudio en el que se discuten aspectos micológicos e inmunológicos, además, por lo que se refiere a las peculiaridades clínicas de esta forma epidémica, ALARCÓN (1957) publicó un análisis con motivo de dos casos que observó en cinco sujetos que penetraron a una gruta cercana a la Ciudad de Monterrey, Estado de Nuevo León.

Como en la coccidioidomicosis también en la histoplasmosis se han practicado exploraciones cutáneas para fijar las zonas de endemicidad del padecimiento. Entre estos estudios se encuentran el realizado por GONZÁLEZ OCHOA *et al.* (1948) en la población de Abalá, Estado de Yucatán, estudio que se llevó paralelamente con pruebas cutáneas a la coccidioidina y con radiografía de torax; los autores encontraron en 150 casos explorados un 55.3% de reactores a la histoplasmina, 23.3% a la tuberculina y ninguno positivo a la coccidioidina; la radiografía del torax mostró en sujetos histoplasmino positivos y tuberculino negativos tres casos de calcificaciones pulmonares múltiples y un caso febril con lesiones parenquimatosas excavadas negativo a bacilo de Koch. MEANA *et al.* (1949) efectúan un estudio similar en mineros y escolares de la población de Taxco, Estado de Guerrero; sus resultados mostraron 69.8% de reacciones positivas entre 563 mineros y 35.7% en 208 niños; en forma simultánea se practicaron la reacción de Mantoux y radiografía de campos pulmonares, habiéndose encontrado que la mayoría de los individuos positivos a la histoplasmina y negativos a la tuberculina presentaban, en la radiografía, multitud de calcificaciones parenquimatosas, pero en ninguno de esos casos se observaron lesiones evolutivas. OCHOA MARTÍNEZ & SANTOSCOY (1951) en una serie de pruebas cutáneas con histoplasmina practicadas en el Estado de Jalisco encontraron 16.4% de reacciones positivas entre 667 sujetos explorados. LÓPEZ SANABRIA (1952) en estudio similar realizado en León, Estado de Guanajuato, encontró que el 30.5% de 200 sujetos acusaron positividad. HERRERA (1952) investiga también la reactividad cutánea a la histoplasmina y tuberculina en 225 mineros en Chihuahua y encuentra que el 20% de las personas estudiadas reaccionan en forma positiva a la histoplasmina únicamente, mientras que el 32.8% reaccionan en forma positiva, tanto a la histoplasmina como a la tuberculina. Finalmente, SLIM VILLEGAS & ARANDA (1953) investigan la frecuencia en 6141 sujetos, en Mexicali, Estado de Baja California Norte, los que acusaron una positividad del 21.5%.

VÁZQUEZ HOYOS (1953) estudia comparativamente las reacciones de precipitación y fijación de complemento en la histoplasmosis experimental del conejo inoculado con la fase levaduriforme de *Histoplasma capsulatum*; como resultado se encuentra que cuando se inoculan células viables al conejo, las reacciones de precipitación y fijación de complemento se comportan en forma similar; sin embargo; cuando el animal se inocula con células muertas de la fase levaduriforme del hongo, la reacción de fijación de complenento da resultados positivos, mientras que la reacción de precipitación no, practicándolas a diferentes tiempos después de la inoculación en ambos casos.

La susceptibilidad comparada entre el hamster y el ratón a la infección por *H. capsulatum* ha sido estudiada por GONZÁLEZ OCHOA & NAVARRETE (1956) encontrándose que el hamster es considerablemente más sensible que el ratón cuando se emplean dosis de inoculación similares; se sugiere que el método puede ser empleado para el diagnóstico en la primoinfección o en las formas leves de histoplasmosis, en las cuales frecuentemente es difícil demostrar el parásito por los procedimiento habituales de laboratorio.

BLASTOMICOSIS

Los nombres de blastomicosis norteamericana, sudamericana y europea tienen suficiente arraigo para ser conservados,sobre todo los dos primeros, aunque únicamente el primero justifique su connotación geográfica. Estos tres padecimientos han sido observados en México aunque a la fecha son muy escasas las publicaciones que tratan el tema en sus diversos aspectos y puede decirse que se reducen a la comunicación de algunos casos; esas publicaciones son insuficientes de ahí que sean necesarios nuevos estudios y la notificación sistemática de los casos que se presenten para conocer la distribución y frecuencia que esas micosis tengan en México.

Blastomicosis Norteamericana

Aunque frecuentemente se considera a México como un país centroamericano, geográficamente pertenece a América del Norte, y en México se ha observado el padecimiento. El primer caso comunicado por MARTÍNEZ BÁEZ *et al* (1954) fué un caso fatal en el cual los exámenes histopatológicos y el cultivo revelaron la presencia de *Blastomyces dermatitidis*; en la publicación se indica que el enfermo había residido por espacio de ocho meses en los Estados Unidos de Norteamérica; sin embargo, entre esa residencia y la aparición de la sintomatología transcurrieron cuatro años por lo que los autores se inclinan a pensar que se trata de un caso mexicano autóctono. Posteriormente hemos tenido conocimiento de dos casos más que no fueron publicados y que correspondían a individuos que no habían salido del país [1]).

Blastomicosis Sudamericana.

El primer caso observado en nuestro país fué comunicado por GONZÁLEZ OCHOA & ESQUIVEL (1950); a partir de esa fecha se han seguido observando otros, algunos permanecen inéditos. El hecho de que este padecimiento exista en México resta valor al término de blastomicosis sudamericana GONZÁLEZ OCHOA & DOMÍNGUEZ (1957b) notifican dos casos más en los que la iniciación del padecimiento fué pulmonar y años después en uno y meses después en el otro, aparecieron las lesiones tegumentarias; en su trabajo los autores hacen comentarios sobre la patogenia de la infección por *Blastomyces brasiliensis*, el tratamiento y la distribución geográfica, ya que estos dos casos así como el primero provenían de una misma área geográfica restringida

Criptococosis o Blastomicosis Europea.

Se cuenta con datos suficientes para considerar a esta blastomicosis como cosmopolita, por consecuencia el llamarle blastomicosis europea de ninguna manera se justifica. Varios casos han sido observados en México, pero la única mención escrita es la de GONZÁLEZ OCHOA (1955a) quien en un trabajo sobre las micosis observadas en México únicamente notifica su existencia. DE BUEN *et al.* (1954) publicaron un trabajo sobre criptococosis ocular, pero el material utilizado no pertenece a enfermos mexicanos.

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[1]) Casos de Medina, San Luis Potosí, S. L. P. y de Del Valle y Alvarado, Torreón, Coah. (Datos no publicados).

En relación con *Cryptococcus neoformans*, GONZÁLEZ OCHOA *et al.* (1950) describen el efecto estimulante que las sulfonamidas-cobre tienen sobre el crecimiento de esa levadura.

RINOSPORIDIOSIS

MENDIOLA & CORTÉS (1950) describieron el único caso de que se tiene conocimiento en México, se trataba de un individuo de sexo masculino residente en una zona lacuestre de la costa del Estado de Jalisco.

ASPERGILOSIS

A pesar de que en algunos países se considera esta micosis como un padecimiento de relativa frecuencia, en México sólo existe una referencia al respecto, un caso de localización pulmonar proveniente del Estado de Colima, mencionado por GONZÁLEZ OCHOA (1951).

TEMAS DIVERSOS

En esta sección se agrupan una serie de trabajos de índole general, tales como revisiones, estudios de conjunto sobre varios hongos, ensayos sobre substancias quimioterapéuticas y otros temas que no ha sido posible clasificar en ninguna de las secciones anteriores.

Desde hace más de 15 años se han llevado a cabo estudios tendientes a conocer los hongos que se encuentran en la atmósfera de la Ciudad de México, dada la importancia que las esporas de éstos tienen como agentes sensibilizantes; entre los trabajos publicados a este respecto se encuentran los de GONZÁLEZ OCHOA & OROZCO (1943) y los más recientes de CUEVA & MONTIEL (1955) y CUEVA *et al* (1958a) (1958b); en ellos se estudia tanto a los hongos que se encuentran en el aire como los de la tierra y el polvo de las habitaciones. Los resultados que obtienen los autores mencionados coinciden en señalar, con ligeras variaciones, que los hongos más frecuentes en la atmósfera y en la tierra de la Ciudad de México son *Penicillium*, *Hormodendrum*, *Alternaria* y *Monilia sitophila*.

BLACKALLER (1952) realiza un estudio similar en la Ciudad de Guadalajara, estado de Jalisco y encuentra que los hongos más frecuentes en la atmósfera son *Hormodendrum*, *Alternaria* y diversas especies de *Aspergillus* y *Penicillium*. Posteriormente este mismo autor (BLACKALLER, 1955) extiende sus observaciones y realiza estudios similares en la región medio occidental de la República Mexicana, encontrando otra vez que las especies que se aislan con más frecuencia corresponden a los géneros *Hormodendrum*, *Alternaria*, *Aspergillus*, *Penicillium* y *Helminthosporium*, siguiéndoles otros en menor proporción. Finalmente, OROPEZA (1958) estudia este mismo tema en la Ciudad de Monterrey, estado de Nuevo León y observa que los géneros más comúnmente aislados son *Alternaria, Hormodendrum, Penicillium* y *Aspergillus,* los cuales predominan durante la estación templada del año (de octubre a marzo).

González Ochoa *et al* (1946) prueban la acción de la penicilina sobre 76 cepas de hongos patógenos y encuentran que con excepción de *Actinomyces bovis,* que resultó sensible hasta una concentración de 0.08μ/ml del antibiótico, los demás hongos se comportan como totalmente resistentes.

González Ochoa & Bojalil (1950) estudian la acción *in vitro* de complejos sulfa-cobre sobre diversos hongos patógenos y encuentran que la sulfamerazina-cobre y el sulfatiazol-cobre tienen actividad sobre *Hormodendrum pedrosoi* y *Nocardia brasiliensis* respectivamente; el resto de las especies estudiadas (*C. albicans, E. floccosum* y *B. dermatitidis*) no mostraron inhibición por concentraciones terapéuticas.

La acción de diversos antibióticos, entre los que se incluyen algunos de uso común, contra diversos hongos productores de micosis profundas, fué estudiada por González Ochoa & Romero (1953) observando que ningún hongo patógeno causante de micosis superficiales o profundas fué sensible a la acción de los antibióticos, con excepción de *A. bovis.* Es menester recordar que el trabajo mencionado fué realizado antes de la intoducción en la clínica de la nystatina, la anfotericina B y la griseofulvina.

El aspecto histopatológico de las micosis profundas ha sido estudiado por Novales (1956) en un artículo en que se describen las peculiaridades histológicas de las lesiones causadas por los hongos, fundamentalmente de aquellas que se observan con más frecuencia en nuestro medio. El diagnóstico de laboratorio de las micosis ha sido tratado en forma resumida por González Ochoa (1956c).

Existen diversas publicaciones que se ocupan de las micosis profundas en general, pero con especial referencia al problema en México (González Chávez, 1946; Aceves Ortega, 1956b y González Ochoa, 1955a) en dichas publicaciones se estudia la frecuencia en nuestro medio de las micosis mencionadas y se hacen consideraciones sobre diagnóstico clínico, diagnóstico de laboratorio, patología y patogenia, agentes etiológicos y terapéutica; en general, puede decirse que los distintos autores coinciden en señalar al micetoma actinomicótico, la esporotricosis, la coccidioidomicosis y la cromomicosis como las micosis profundas más frecuentes en nuestro medio.

Por otra parte, diversos autores han estudiado diferentes micosis agrupadas desde el punto de vista de su localización anatómica; así, González Ochoa (1951) presenta un trabajo de tipo monográfico sobre las micosis pulmonares en el que se describen con detalle, entre otras cosas, las diversas enfermedades de esta clase que habían sido observadas en México hasta la fecha de la publicación. Castro Villagrama *et al* (1956) discuten la patología ósea y el aspecto radiológico de las lesiones producidas por el micetoma actinomicótico, la esporotricosis y la coccidioidomicosis. En relación también con las lesiones óseas producidas por los hongos que causan las micosis profundas, Romo Diez (1957) hace consideraciones sobre el tratamiento médico y quirúrgico de dichas lesiones y comunica sus observaciones sobre algunos casos estudiados por él. Las micosis de localización ocular han sido estudiadas por González Ochoa (1953b) desde un punto de vista clínico y terapéutico, señalándose también cuales de ellas son las más frecuentes en México.

Finalmente, González Ochoa (1956a) ha propuesto una clasificación clínica de las micosis basada en la particular afinidad que presentan los hongos patógenos por el tegumento cutáneo mucoso, y la manera de como esos hongos producen la manifestación tegumentaria; con esta base patogénica considera tres grupos: 1) Micosis Exclusivamente Tegumentarias, 2) Micosis Inicialmente Tegumentarias, y 3) Micosis Secundariamente Tegumentarias. En el primer grupo, al que corresponden las llamadas micosis superficiales, se colocan aquellos padecimientos en que el agente patógeno no penetra más allá de las estructuras superficiales de la piel y mucosas y que además invade las faneras de la piel. En el segundo grupo se colocan las micosis en las que el hongo penetra inicialmente por el tegumento cutáneo-mucoso, pero su agresión va más allá de la epidermis; este grupo comprendería la moniliasis superficial, la cromomicosis, la rinosporidiosis, la esporotricosis y el micetoma. En el tercer grupo quedan incluídas aquellas micosis en que la lesión inicial es profunda, preferentemente visceral, y de ésta sobre todo pulmonar, en estas micosis posteriormente, tarde o temprano, en función del hongo y de la sobre vida del enfermo, se producirían las manifestaciones tegumentarias; en este tercer grupo se colocan el resto de las micosis, o sea la histoplasmosis, coccidioidomicosis, las blastomicosis, la actinomicosis, la moniliasis metastásica y la nocardiasis, ésta última con muy poca o ninguna tendencia a exteriorizarse en las localizaciones pulmonar y meningoencefálica.

APENDICE

Numerosos son los trabajos que se han realizado en México en relación con otros campos de la Micología (fitopatología, antibióticos, taxonomía, micología industrial y fisiología de los hongos). Tales publicaciones no han podido ser mencionadas, primero por la índole del tema que se ha tratado y segundo por la extensión que alcanzaría esta comunicación.

Para terminar, los autores agradecen al Profesor Tibor Benedek la invitación hecha para presentar este trabajo y a la Dra. Yolanda Ortiz del Servicio de Dermatología del Hospital General de

México, el habernos proporcionado la facilidad de consultar algunas de las referencias bibliográficas que se mencionan a lo largo del texto.

RESUMEN

Se presenta una revisión general de algunas de las publicaciones mexicanas sobre micología médica aparecidas durante los años de 1946 a 1958. Se divide el trabajo en secciones de acuerdo con las micosis que se han observado en México y se comentan los trabajos publicados correspondientes a cada tema.

Se observa que las micosis más frecuentes en México, además de las dermatomicosis, son el micetoma actinomicósico producido por *Nocardia brasiliensis*, la esporotricosis, la coccidioidomicosis, la actinomicosis, la cromomicosis y la infección primaria pulmonar epidémica por *H. capsulatum*. Las demás se presentan en forma escasa y sólo en ocasiones se encuentran comunicaciones en la literatura.

Se proporciona la bibliografía de los trabajos comentados.

SUMMARY

A general review is presented of some of the mexican literature dealing with medical mycology appeared during the 1946—1958 period.

This paper is divided in several sections in accordance with the mycoses that have been observed in Mexico; brief comments are made of each reference.

It can be observed that in addition to the dermatomycosis, the most frequent mycoses in Mexico are the actinomycotic mycetoma produced by *Nocardia brasiliensis*, sporotrichosis, coccidioidomycosis, actinomycosis, chromomycosis and the acute pulmonar primoinfection of histoplasmosis. The other mycoses are not very common in Mexico and are only occasionally observed.

A complete list of the reviewed papers is appended.

Bibliografia

ACEVES ORTEGA, R. (1950) La esporotricosis. Bol. Soc. Med. Guadalajara. **22:** 243—245.

ACEVES ORTEGA, R. (1956a) Algunas observaciones efectuadas en 1200 casos de tiñas de la cabeza tratadas con acetato de talio de 1949 a 1956. Mem. III Congr. Iber. Latinoamer. Dermatol. pp. 171—174, México.

ACEVES ORTEGA, R. (1956b) Las micosis profundas en nuestro medio. Mem. III Congr. Iber. Latinoamer. Dermatol. pp. 239—243. México.

ACEVES ORTEGA, R. (1958) Nocardiosis. Sem. Méd. Mex. **17:** 401—407.

ALARCON, D. G. (1957) Histoplasmosis pulmonar epidémica. Gac. Méd. Méx. **87:** 745—750.

BARBA RUBIO, J. & G. PEREZ SUAREZ. (1956) El acetato de talio en el tratamiento de la tiña. Revisión de nuestra experiencia en 13 años. Mem. III Congr. Iber. Latinoamer. Dermatol. pp. 168—170. México.

BLACKALLER, A. (1952) Contribución al conocimiento de la flora micológica de la Cd. de Guadalajara, Jal. Rev. Mex. Alergología. **3:** 12—14.

BLACKALLER, A. (1955) Hongos atmosféricos en la región medio occidental de la República Mexicana. Alergia. **2:** 148—156.

Bojalil, L. F. & J. A. Shiels. (1950) Estudio de la acción in *vitro* de algunos compuestos sobre el crecimiento de varias especies de *Nocardia*. Rev. Palud. Med. Trop. **2:** 133—136.

Brumpt, E. (1949) Précis de Parasitologie. 6a. ed. Masson et Cie. editores. París. Vol. II, pp. 1688—1691.

Cardona, A. & T. Velazquez. (1954) Infección del sistema nervioso central por *Coccidioides immitis*. Rev. Inst. Salub. Enfs. Trops. (Méx.) **14:** 179—188.

Castro Villagrana, J., A. Castanedo, I. Ugalde, M. Garcia, I. Navarro, A. Gonzalez-Ochoa & S. Lopez. (1956) Las micosis óseas. Rev. Cir. (Méx.) **7:** 271—176.

Cicero, R. E. (1918) Comunicación a la Academia de Medicina de México. (Citado por González Urueña, J., 1928).

Cueva, J. & J. Montiel. (1955) Hongos de la tierra en la Ciudad de México y su relación con las enfermedades alérgicas. Alergia. **2:** 93—103.

Cueva, J., A. G. Tellez, L. Ortiz & Y. Castillo. (1958a) Hongos del interior de las habitaciones de la Ciudad de México. Alergia. **6:** 253—257.

Cueva, J., A. G. Tellez, L. Ortiz & Y. Castillo. (1958b) Hongos atmosféricos de la Ciudad de México. Rev. Méd. Hosp. Gral. **21:** 511—521.

De Buen, S., L. E. Zimmerman & H. C. Foerster. (1954) Patología ocular de la criptococosis. Rev. Inst. Salub. Enfs. Trops. (Méx.) **14:** 163—172.

De La Garza, S. & M. A. Rodriguez. (1952) Cuatro casos de coccidioidomicosis diagnosticados por biopsia y comprobados por cultivo. Rev. Hosp. Universitario (Monterrey, N. L.) **2:** 181—214.

Durazo, F. (1956) Moniliasis en urología. Rev. Urol. **14:** 162—167.

Estrada, C. & P. Lavalle. (1956) Tiña imbricada. Tratamiento con benzotiazol. Mem. III Congr. Iber. Latinoamer. Dermatol. pp. 176—178, México.

Garcia, M. (1950) Sulfonas en el tratamiento del micetoma. Estudio de un caso. Pren. Méd. Méx. **15:** 262—266.

Germes Leal, O. (1953) Micosis superficiales. Bol. Cent. Méd. Chihuahua. **4:** (23), 21—31.

Gonzalez Benavides, J. (1952) La esporotricosis como enfermedad ocupacional de los alfareros. Rev. Hosp. Universitario (Monterrey, N. L.). **2:** 215—232.

Gonzalez Chavez, A. (1946) Las micosis profundas más comunes en México. Rev. Méd. Hosp. Gral. **8:** 1018—1026.

Gonzalez Herrejon, S. (1919) El acetato de talio en el tratamiento de las tiñas. Tesis profesional (Médico Cirujano) México.

Gonzalez-Mendoza, A. & Bojalil, L. F. (1958) The incidence of *Candida albicans* in the sputum of tuberculous patients. Am. Rev. Tuberc. **77:** 543—545.

Gonzalez Ochoa, A. (1941) Hallazgo de *Fonsecaea pedrosoi* var. *cladosporioides* en México. Rev. Inst. Salub. Enfs. Trops. (Méx.) **2:** 187—191.

Gonzalez Ochoa, A. (1949) Coccidioidomicosis. Algunos conceptos actuales del padecimiento con especial mención al problema mexicano. Pren. Méd. Méx. **14:** 246—252.

Gonzalez Ochoa, A. (1951) Las micosis pulmonares en México. Mem. IV Congr. Nac. Tuberc. Silicosis. pp. 247—345, México.

Gonzalez Ochoa, A. (1953a) El micetoma tóraco-pulmonar por *Actinomyces bovis* y *Nocardia brasiliensis*. Gac. Méd. Méx. **83:** 109—115.

Gonzalez Ochoa, A. (1953b) Micosis oculares. Pren. Méd. Méx. **18:** 30—35.

Gonzalez Ochoa, A. (1954) Recientes adquisiciones en el conocimiento de la coccidioidomicosis. Gac. Méd. Méx. **84:** 307—312.

Gonzalez Ochoa, A. (1955a) Las enfermedades por hongos en México. Rev. Inst. Salub. Enfs. Trops. (Méx.) **15:** 133—147.

Gonzalez Ochoa, A. (1955b) El problema de las dermatomicosis en México. I. Las tiñas de la piel lampiña. Rev. Méd. Sria. Marina. **1:** 15—31.

Gonzalez Ochoa, A. (1956a) Clasificación clínica de las micosis. Rev. Inst. Salub. Enfs. Trops. (Méx.) **16:** (3); 1—8.

Gonzalez Ochoa, A. (1956b) Pitiriasis versicolor. Rev. Méd. Sria. Marina. **2:** 81—88

Gonzalez Ochoa, A. (1956c) Diagnóstico de laboratorio de las micosis. Rev. Mex. Lab. Clín. **8:** 17—22.

Gonzalez Ochoa, A. (1957a) Histoplasmosis pulmonar aguda primaria. Gac. Méd. Méx. **87:** 733—744.

GONZALEZ OCHOA, A. (1957b) Moniliasis y monilias. Rev. Inst. Salub. Enfs. Trops. (Méx.) **17:** 13—21.

GONZALEZ OCHOA, A. (1957c) Deep Mycoses due to Actinomycetes (Milestones in Dermatology). Excerpta Medica. Secc. XIII. Dermatology and Venereology. **11:** 371—372.

GONZALEZ OCHOA, A. & M. AHUMADA. (1958) Tratamiento del micetoma actinomicótico por la inyección local de diamino-difenilsulfona. Rev. Inst. Salub. Enfs. Trops. (Méx.) **18:** 41—44.

GONZALEZ OCHOA, A. & F. BARANDA. (1953) Una prueba cutánea para el diagnóstico del micetoma actinomicótico por *Nocardia brasiliensis*. Rev. Inst. Salub. Enfs. Trops. (Méx.) **13:** 189—197.

GONZALEZ OCHOA, A. & L. F. BOJALIL. (1950) Actividad *in vitro* de complejos sulfa-cobre, sobre algunos hongos patógenos. Rev. Inst. Salub. Enfs. Trops. (Méx.) **11:** 79—91.

GONZALEZ OCHOA, A. & J. CORDOVA. (1957) El factor sensibilización en las dermatofitosis de la piel lampiña. Rev. Inst. Salub. Enfs. Trops. (Méx.). **17:** 107—113.

GONZALEZ OCHOA, A. & L. DOMINGUEZ. (1957a) Algunas observaciones epidemiológicas y patogénicas sobre la moniliasis oral en el recién nacido. Rev. Inst. Salub. Enfs. Trops. (Méx.) **17:** 1—12.

GONZALEZ OCHOA, A. & L. DOMINGUEZ. (1957b) Blastomicosis sudamericana. Casos mexicanos. Rev. Inst. Salub. Enfs. Trops. (Méx.) **17:** 97—106.

GONZALEZ OCHOA, A. & E. ESQUIVEL. (1950) Primer caso de granuloma paracoccidioideo (Blastomicosis sud-americana) en México. Rev. Méd. Hosp. Gral. **13:** 159—168.

GONZALEZ OCHOA, A. & P. LAVALLE. (1947) Dermatofitos causantes de las diversas tiñas de la piel lampiña observadas en nuestro medio. Rev. Inst. Salub. y Enfs. Trops. (Méx.) **8:** 265—272.

GONZALEZ OCHOA, A. & E. MACOTELA. (1955) Tratamiento de las micosis profundas con esteroides. Inutilidad de la pregnenolona en un caso de micetoma por *Nocardia brasiliensis*. Rev. Inst. Salub. Enfs. Trops. (Méx.) **15:** 9—14.

GONZALEZ OCHOA, A. & R. MARCOR. (1952) Inoculación de *Coccidioides immitis* Stiles, 1896 al embrión de pollo. Rev. Soc. Mex. Hist. Nat. **13:** 283—297.

GONZALEZ OCHOA, A. & F. NAVARRETE. (1956) Susceptibilidad comparada entre el hamster y el ratón a la infección por *Histoplasma capsulatum*. Rev. Inst. Salub. Enfs. Trops. (Méx.) **16:** (3); 9—15.

GONZALEZ OCHOA, A. & C. OROZCO. (1943) Los hongos del aire en la Ciudad de México. Sus relaciones con los factores atmosféricos. Rev. Inst. Salub. Enfs. Trops. (Méx.) **4:** 259—264.

GONZALEZ OCHOA, A. & C. OROZCO. (1953) El micetoma maduromicósico en México, Mem. Congr. Cient. Mex. **10:** 266—270.

GONZALEZ OCHOA, A. & C. OROZCO. (1957) Dermatofitos causantes de la *tinea unguis* en México. Rev. Inst. Salub. Enferm. Trops. (Méx.) **17:** 93—95.

GONZALEZ OCHOA, A. & H. ROSILES. (1953) Probable curación de un caso de granuloma coccidioidomicósico por la hidroxicloroquima. Rev. Inst. Salub. Enfs. Trops. (Méx.) **13:** 261—263.

GONZALEZ OCHOA, A. & A. ROMERO. (1953) Acción de algunos antibióticos contra los principales hongos patógenos. Mem. Congr. Cient. Mex. **10:** 307—308.

GONZALEZ OCHOA, A. & B. ROMO (1945) Dermatofitos causantes de tiña de la piel cabelluda en la Ciudad de México. Rev. Inst. Salub. Enfs. Trops. (Méx.) **6:** 145—148.

GONZALEZ OCHOA, A. & R. SALINAS. (1953) Algunos aspectos immunológicos de la esporotricosis humana y experimental. Mem. Congr. Cient. Mex. **10:** 300—306.

GONZALEZ OCHOA, A. & M. A. SANDOVAL. (1949) Susceptibilidad de *Actinomyces bovis* a la sulfadiazina, penicilina, estreptomicina y promina. Rev. Inst. Salub. Enfs. Trops. (Méx.) **10:** 69—76.

GONZALEZ OCHOA, A. & M. A. SANDOVAL. (1955) Características de los actinomycetes patógenos más comunes. Rev. Inst. Salub. Enfs. Trops. (Méx.) **15:** 149—161.

GONZALEZ OCHOA, A. & M. A. SANDOVAL. (1956a) Levaduras en padecimientos intestinales no tratados con antibióticos. Rev. Inst. Salub. Enfs. Trops. (Méx.) **16:** (4); 15—19.

GONZALEZ OCHOA, A. & M. A. SANDOVAL. (1956b) Revisión determinativa de algunas especies de actinomycetes patógenos descritas como diferentes. Rev. Inst. Salub. Enfs. Trops. (Méx.) **16:** (3); 17—25.

GONZALEZ OCHOA, A. & E. SOTO FIGUEROA. (1947) Polisacáridos de *Sporotrichum schenckii*. Rev. Inst. Salub. Enfs. Trops. (Méx.) **8:** 143—153.

GONZALEZ OCHOA, A. & R. SOTO PACHECO. (1950) Desarrollo de *Sporotrichum schenckii* en el pus obtenido de gomas esporotricósicas. Rev. Inst. Salub. Enfs. Trops. (Méx.) **11:** 3—19.

GONZALEZ OCHOA, A. & A. VAZQUEZ. (1953) Relaciones serológicas de los principales actinomycetes patógenos. Rev. Inst. Salub. Enfs. Trops. (Méx.) **13:** 177—187.

GONZALEZ OCHOA, A., M. A. SANDOVAL & A. MORENO. (1946) Acción de la penicilina sobre los hongos patógenos. Rev. Inst. Salub. Enfs. Trops. (Méx.) **7:** 193—200.

GONZALEZ OCHOA, A. E. ESQUIVEL & M. CACERES. (1948) Investigación de la reactividad cutánea a la histoplasmina, tuberculina y coccidioidina, relacionada con el catastro torácico en Yucatán. Rev. Inst. Salub. Enfs. Trops. (Méx.) **9:** 55—63.

GONZALEZ OCHOA, A., L. F. BOJALIL & R. SOTO PACHECO. (1950) Acción estimulante de las sulfonamidas-cobre y de la penicilina sobre el desarrollo de *Cryptococcus neoformans* y *Sporotrichum schenckii* respectivamente. Rev. Soc. Mex. Hist. Nat. **11:** 35—41.

GONZALEZ OCHOA, A., J. SHIELS & P. VAZQUEZ. (1952) Acción de la 4.4' diamino-difenil-sulfona frente a *Nocardia brasiliensis* Gac. Méd. Mex. **82:** 345—353.

GONZALEZ OCHOA, A., E. HERNANDEZ & R. COLORADO. 1(953) Experimentación con vainillato de etilo en dos casos de coccidioidomicosis y uno de micetoma actinomicótico por *Nocardia brasiliensis*. Rev. Inst. Salub. Enfs. Trops. (Méx.) **13:** 265—274.

GONZALEZ OCHOA, A., L. DOMINGUEZ & E. MACOTELA. (1955) Tratamiento de la moniliasis oral con nystatin. Rev. Inst. Salub. Enfs. Trops. (Méx.) **15:** 195—203

GONZALEZ URUEÑA, J. (1928) Le traitement des teignes par l'acétate de thallium. Masson et Cie. Editores. París.

GUERRERO SANTOS, J. (1956) Tratamiento quirúrgico de la cromomicosis. Mem. III Congr. Iber. Latinoamer. Dermatol. p. 181, México.

HAYASHI BECERRA, E. (1952) El acetato de talio en las tiñas de la cabeza. Rev. Soc. Méd. Tapachula. **1:** 106—110.

HERRERA, A. G. (1952) Investigación de la reactividad cutánea a la histoplasmina y tuberculina en un grupo de obreros del mineral de Aquiles Serdán, Chih. Mem. IV Nac. Tuberc. Silic. 347—355. San Luis Potosí, México.

LATAPI, F. (1950) Esporotricosis facial infantil. Nota clínica. Pren. Méd. Mex. **15:** 259—262.

LATAPI, F. (1956) Micetoma. Análisis de 100 casos estudiados en la Ciudad de México. Mem. III Congr. Iber. Latinoamer. Dermatol. pp. 203—213.

LAVALLE, P. (1956a) Esporotricosis. Algunas peculiaridades mexicanas y síntesis patogénica. Mem. III Congr. Iber. Latinoamer. Dermatol. pp. 190—197, México.

LAVALLE, P. (1956b) Coccidioidomicosis en México Mem. III Congr. Iber. Latinoamer. Dermatol. pp. 198—202, México.

LAVALLE, P. & J. MILLAN. (1949) Estudio clínico y micológico de un caso de micetoma de la mano. Pren. Méd. Mex. **14:** 106—107.

LOPEZ SANABRIA, J. M. (1952) Incidencia de reacciones positivas a la tuberculina, histoplasmina y coccidioidina en León, Gto. Rev. Mex. Alergología. **3:** 15—16.

MADRID, G. (1948) Las micosis pulmonares. (la. parte). Rev. Mex. Tuberc. **9:** 32—55.

MADRID, G. (1950) La resección pulmonar en el tratamiento de la coccidioidomicosis Prensa Médica (Hermosillo, Son.). **10:** 2—11.

MARTINEZ BAEZ, M. (1940) Un caso de probable dermatitis verrucosa. Estudio histopatológico. Rev. Inst. Salub. Enfs. Trops. (Méx.) **1:** 323—337.

MARTINEZ BAEZ, M., A. REYES & A. GONZALEZ-OCHOA. (1954) Blastomicosis norteamericana en México, Rev. Inst. Salub. Enfs. Trops. (Méx.) **14:** 225—232.

MEANA, J. B., C. RODRIGUEZ & F. PRUNEDA. (1949) Investigación de la reactividad cutánea a la tuberculina, histoplasmina y esporotricina en 563 mineros. Mem. VII. Congr. Panam. Tuberc. y II Nac. Tuberc. y Silicosis. pp. 761—769. México.

MENDIOLA, R. (1947) Maduromicosis por *Monosporium apiospermum*. Mem. I Congr. Mex. Méd. Vol. II. 257—260. México.

MENDIOLA, R. & R. CORTES (1950) Rinosporidiosis. Primer caso encontrado en México. Rev. Inst. Salub. Enfs. Trops. (Méx.) **11:** 159—165.

MENDIOLA, R. & J. M. LICONA. (1953) Un caso de histoplasmosis. Rev. Inst. Salub. Enfs. Trops. (Méx.). **13:** 9—12.

MOORE, M. (1941) The chorio-allantoic membrane of the developing chick as a medium for the cultivation and histopathologic study of pathogenic fungi. Amer. J. Path. **17:** 103—120.

NOVALES, J. (1956) Histopatología de las micosis profundas. Mem. III Congr. Iber. Latinoamer. Dermatol. pp. 222—226, México.

NOVALES, J. (1958) Cromomicosis. Comunicación de un caso. Dermatología. Rev. Mex. **2:** 30—36.

NUÑEZ ANDRADE, R. (1953) Tiña imbricada. Medicina (Méx.) **33:** 473—479.

OCHOA MARTINEZ, I. & G. SANTOSCOY (1951) Histoplasmosis y coccidioidomicosis en el Estado de Jalisco. IV Congr. de tuberc. y Silicosis. San Luis Potosí, S. L. P. México.

OROPEZA, A. (1958) Hongos anemófilos en la cíudad de Monterrey, N. L. Alergia **5:** 111—121.

PEREZ-REYES, R. & M. A. LARRE. (1951) Primer caso de coccidioidomicosis en el Estado de Michoacán, México. Rev. Palud. Med. Trop. **3:** 125—128.

PERRIN, T. G. & M. MARTINEZ BAEZ. (1943) Nota sobre el primer caso de histoplasmosis en México. Rev. Inst. Salub. Enfs. Trops. (Méx.) **4:** 79—87.

QUIJANO, M. (1958) Moniliasis en cirugía general. Pren. Méd. Mex. **23:** 340—341.

RODRIGUEZ, O. (1956) Cromoblastomicosis, su tratamiento con calciferol. Mem. III Congr. Iber. Latinoamer. Dermatol. pp. 182—189, México.

ROMO DIEZ, X. (1955) Contribución al estudio de la nocardiosis. Rev. Méd. Hosp. Gral. **18:** 401—413.

ROMO DIEZ, X. (1957) Micosis profundas, lesiones óseas y su tratamiento quirúrgico. Rev. Méd. Hosp. Gral. **20:** 97—111.

SALAZAR MALLEN, M. (1957) Importancia de los antígenos de *Candida* y *Monilia* desde el punto de vista de la alergología clínica. Alergia. **4:** 407—110.

SANCHEZ MARROQUIN, A. (1947) Estado actual de los conocimientos acerca de la esporotricosis, Ciencia (Méx.) **8:** 25—34.

SANCHEZ MARROQUIN, A. (1958) Taxonomic considerations on some *Candida* yeasts. Rev. Latinoamer. Microbiol. **1:** 233—242.

SCHNAAS, G., A. GONZALEZ-OCHOA & MARTINEZ-BAEZ. (1957) Coccidioidomicosis natural en el perro. Cienc. Vet. **2:** 417—421.

SCHNAAS, G. & A. GONZALEZ OCHOA. (1958) Aislamiento de *Microsporum gypseum* en un perro con tiña. Cienc. Vet. **3:** 1—2.

SIMONS, R. D. G. PH. (Ed.) (1952) A Handbook of Tropical Dermatology. 2 Vols. Elsevier Publishing Co. Amsterdam.

SLIM VILLEGAS, V. J. & B. ARANDA (1953) Coccidioidomicosis e histoplasmosis en el Valle de Mexicali, Bol. Of. Sanit. Panamer. **34:** 137—142.

VAZQUEZ HOYOS, A. (1953) Estudio comparativo entre las reacciones de precipitación y fijación de complemento en la histoplasmosis experimental. Mem. Congr. Cient. Mex. **10:** 295—299.

IN MEMORIAM

DR. TAKEWO HEMMI (1889—1959)

Professor TAKEWO HEMMI passed away on October 12, 1959 at his home in Kyoto after a period of prolonged illness. He had been on leave of absence from his institute of the Osaka prefectural University, his last post held, since March 1957, due to his ill health.

Born in Takaoke, northern part of Japan in 1889, he graduated in Agriculture from the Tohoku University, Sapporo in 1915; thus, he began his career as a plant pathologist. His early training was received at the Hokkaido University, Sapporo under the direction of Professor KINGO MIYABE, who is the founder of plant pathology in Japan. In 1920 he received his doctor's degree from the latter university after completing the post graduate work there.

His early interest was in the anthracnose diseases of plants. He collected many Japanese specimens and clarified the relation of mycelial growth and pathogenicity of the causal fungi to the disease outbreak.

Prior to the appointment as professor of Kyoto University in 1924, and Director of the Laboratory of Plant Pathology, he went to America and studied epidemiology of plants at the University of Wisconsin under the direction of Professor L. R. JONES.

Throughout his career, his interest was in the problems dealing with the epidemics of rice plant diseases; and in this field are found many of his accomplished contributions, where considerable emphasis is particulary notable on the subject of blast disease. He clarified various physiological characteristics of the fungus, *Piricularia oryzae*, and the relation of environmental factors, particularly the cultural practices of the rice plants, to the occurrence of the blast disease. It may safely be said that he was largely responsible for the basis of present control measures of rice blast disease in Japan. His studies included also the silicification of epidermal cell walls in the leaves of rice plants as concerning the blast-resistance, and the induced resistance of rice plants against blast disease by treating the seeds and seedlings with chemicals, such as the plant growth substances. These contributions were included in the book written in Japanese entitled the "Studies on Blast Disease" published by the Asakura Shoten, Tokyo in 1949. Diseases of trees and wood-rotting fungi were also included in Dr. HEMMI'S interest.

Dr. HEMMI was also a mycologist. His collections were vast in *Septoria*, anthracnose fungi and wood-rotting fungi. Protomycetes and mushrooms were also included. Although he did not publish

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any papers on *Protomyces*, he made abundant observations on clarifying the life cycle of this fungus.

From 1928 to 1929 and from 1946 to 1950 he was president of the Japanese Phytopathological Society, and was made Honorary Member of the society in 1952. He was also on the editorial board of Mycopathologia et Mycologia Applicata and of "Phytopathologische Zeitschrift". During 1931 and 1937, he edited the journal, Shokubutsu Byogai Kenkyu (Forschungen auf dem Gebiet der Pflanzenkrankheiten), in which he published many articles of experiments conducted in his laboratory.

After he left the Kyoto University in 1949, he accepted the call as a professor of Osaka Prefectural University in 1952, where he taught plant pathology until the time he became ill. He was one of the most distinguished scholars of his time, and was known as a man of strong conviction and steadfast character.

SHIGEYASU AKAI, Kyoto

PUBLICATIONS OF DR. TAKEWO HEMMI

Articles

1916

On the die-back disease of *Paulownia tomentosa* caused by a new species of *Valsa*. Bot. Mag., Tokyo, **30**(357) : 304—315.

Kurze Mitteilung über einige parasitische Pilze Japans. Bot. Mag., Tokyo, **30**(358) : 334—344.

On a new canker-disease of *Prunus yedoensis*, *P. mume* and on the species caused by *Valsa japonica* Miyabe et Hemmi sp. n. Jour. Coll. Agr., Tohoku Imp. Univ., Sapporo, **7**(4) : 257—319.

1918

Vorläufige Mitteilung über eine Anthraknose von *Evonymus Japonica*. Ann. Phytopath. Soc. Japan, **1**(1): 9—15.

1919

On a disease of some leguminous plants caused by *Ceratophorum setosum* Kirchner. Trans. Sapporo Nat. Hist. Soc., **7**(2) : 116—127.

Vorläufige Mitteilung über eine Anthraknose von *Carthamus tinctorius*. Ann. Phytopath. Soc. Japan, **1**(2) : 1—11.

1920

Beiträge zur Kenntnis der Morphologie und Physiologie der japanischen *Gloeosporium*. Coll. Agr., Hokkaido Imp. Univ., Sapporo, **9**(1) : 1—159.

Kurze Mitteilung über drei Fälle von Anthraknose auf Pflanzen. Ann. Phytopath. Soc. Japan, **1**(3) : 1—9.

1921

Nachträge zur Kenntnis der *Gloeosporien*. Coll. Agr., Hokkaido Imp. Univ., **9**(6) : 305—346.

1922

On the occurrence of *Mycosphaerella* wilt of Muskmelons in Japan. Phytopath., **12** : 394—397.

1923

On the relation of temperature to the damping-off of gardencress seedlings by *Pythium debaryanum* and *Corticium vagum*. Phytopath., **13**(6) : 273—282.

1926

An outline of experimental studies on the "indefinite" diseases of the rice plant. Proc. Third Pan-Pacific Sci. Congr. Tokyo, 2108—2111.

1927

With Hisao Nakamura
Studies on septorioses of plants. I. Comparison of two different species of septoria causing the leaf-spot diseases of the cultivated chrysanthemum. Memoirs Coll. Agr. Kyoto Imp. Univ. **3**: 1—24.

With Tomowo Nojima
Contributions to the knowledge of anthracnoses of plants. I. Notes on three new or little known anthracnoses of the cultivated plants in Japan. Memoirs Coll. Agr. Kyoto Imp. Univ., **3** : 25—39.

An outline of the experimental study on the "indefinite" diseases of the rice plant. Ann. Phytopanth. Soc. Japan, **2**(1) : 9—13.

1928

With Fusataro Seto
Experiments relating to stimulative action by the causal fungus of the "Bakanae" disease of rice. Proc. Imp. Acad., **4**(4) : 181—184.

With Isamu Matsuura
Experiment relating to toxic action by the causal fungus of Helminthosporiose of rice. Proc. Imp. Acad., **4**(4) : 185—187.

With Takuji Abe
An outline of the investigations on the seed- and seedling-rot of rice caused by a water-mould, *Achlya prolifera* Nees. Jap. J. Bot., **4**(2) : 113—123.

With Kuniomi Yokogi
Experimental studies on the pathogenicity of certain fungi on rice seedlings. Memoirs Coll. Agr. Kyoto Imp. Univ., **7** : 1—22.

With Shigeru Endo
On a staining method for testing the viability of sclerotia of fungi. Memoirs Agr. Kyoto Imp. Univ., **7** : 39—49.

With Tomowo Nojima
Studies on *Polyporus orientalis* parasitic on the roots of pine trees (in Japanese). Ann Phytopath. Soc. Japan, **2**(2) : 1—19.

1929

With Shigekatsu Hirayama and Tomowo Nojima
Studies on *Fomes ulmarius* causing the heartwood-rot of *Cryptomeria japonica* (in Japanese). Bot Mag., Tokyo, **43**(516): 657—675.

1931

With Shizuko Kurata
Studies on septorioses of plants. II. *Septoria azaleae* Voglino causing the brown-spot disease of the cultivated azaleas in Japan. Memoirs Coll. Agr. Kyoto Imp. Univ., **13** : 1—22.

With Shizuko Kurata
Notes on three diseases of azaleas. Forsch. a. d. Gebiet d. Pflanzenkrankheiten (Shokubutsu Byogai Kenkyu) **1** : 1—12.

With TAKUJI ABE
On the relation of temperature and period of continuous wetting to the infection of the rice plant by *Piricularia oryzae*. Forsch. a. d. Gebiet d. Pflanzenkrankheiten (Shokubutsu Byogai Kenkyu) **1** : 33—45.

With HASHIO SUZUKI
On the relation of soil moisture to the development of the helminthosporium disease of rice seedlings. Forsch. a. d. Gebiet d. Pflanzenkrankheiten (Shokubutsu Byogai Kenkyu) **1** : 90—98.

With SHIZUKO KURATA
Pathological studies on *Polyporus betulinus* (Bull.) Fr. Forsch. a. d. Gebiet d. Pflanzenkrankheiten (Shokubutsu Byogai Kenkyu) **1** : 206—224.

1932

Notes on some Japanese fungi. Bot. Mag., Tokyo, **46**(544) : 160—168.

Studies on some wood-destroying fungi attacking conifers in Japan. Memoirs Coll. Agr. Kyoto Imp. Univ., **20** 1—29.

With TAKUJI ABE
Studies on rice blast disease. II. (in Japanese). Contr. Improv. Agr., Min. Agr. Forest., **47** : 1—204.

1933

Experimental studies on the relation of environmental factors to the occurrence and severity of blast disease in rice plants. Phytopath., **6**(3) : 305—324.

With TAKUJI ABE
On the relation of air humidity to germination of urediniospores of some species of *Puccinia* parasitic on cereals. Forsch. a. d. Gebiet d. Pflanzenkrankheiten (Shokubutsu Byogai Kenkyu) **2** : 1—9.

With SHIZUKO KURATA
Studies on septorioses of plants. V. *Septoria menthae* (Thüm.) Oud. causing the serious leaf-spot disease of cultivated mints in Japan. Forsch. a. d. Gebiet d. Pflanzenkrankheiten (Shokubutsu Byogai Kenkyu) **2** : 10—19.

On *Stereum induratum* Berk. and *Trametes dickinsii* Berk. causing dry-rot of fagaceous woods. Forsch. a. d. Gebiet d. Pflanzenkrankheiten (Shokubutsu Byogai Kenkyu) **2** : 328—333.

With SHIZUKO KURATA
Miscellaneous notes on Japanese fungi I (in Japanese). Acta Phytotaxono. Geobot., **2**(2) : 109—117.

1934

With SHIZUKO KURATA
Miscellaneous notes on Japanese fungi. II (in Japanese). Acta Phytotaxono. Geobot., **3**(2) : 71—80.

1936

With TAKUJI ABE JUKICHI IKEYA and YOSHITAKA INOUE
Studies on rice blast disease. IV. (in japanese). Contr. Improv. Agr., Min. Agr. Forest., **105** : 1—145.

1937

On cereal diseases in Japan. Forsch. a. d. Gebiet d. Pflanzenkrankheiten (Shokubutsu Byogai Kenkyu) **3** : 1—17.

With SHIZUKO NIWA
On gray-mold neck rot of stored onions. Forsch. a. d. Gebiet d. Pflanzenkrankheiten (Shokubutsu Byogai Kenkyu) **3** : 234—249.

1939

With Jvunzo Imura
On the relation of air-humidity to conidial formation in the rice blast fungus, *Piricularia oryzae*, and the characteristics in the germination of conidia produced by the strains showing different pathogenicity (in Japanese). Ann. Phytopath. Soc. Japan, **9**(3) : 147—156.

With Sentaro Konishi
Studies on the Phytophthora rot of eggplant on the market (in Japanese). Ann. Phytopath. Soc. Japan, **9**(3) : 157—169.

Occurrence of the apple tree canker caused by *Botryosphaeria ribis* in Japan (in Japanese). Ann Phytopath. Soc. Japan. **9** (3) : 184—190.

With Shizuko Niwa
On botrytis rots of stored onions (in Japanese). Ann. Phytopath. Soc. Japan, **8**(4) : 309—326.

1940

Studies on septorioses of plants. VI. *Septoria glycines* Hemmi causing the brown-spot disease of soy bean. Memoirs Coll. Agr. Kyoto Imp. Univ., **47** : 1—14.

1941

With Jyunzo Aoyagi
Germination of the macroconidia of *Gibberella fujikuroi* in relation to some environmental factors (in Japanese). Ann. Phytopath. Soc. Japan, **11**(2) : 66—80.

With Takuji Abe and Yoshitaka Inoue
Studies on rice blast disease. VI. (in Japanese). Contr. Improv. Agr., Min. Agr. Forest., **157** : 1—232.

1942

Studies on rice blast disease. VII. (in Japanese). Contr. Improv. Agr., Min Agr. Forest., **159** : 1—94.

1943

Studies on fungi pathogenic to forest trees in Korea (in Japanese). ActaPhytotaxono. Geobot., **13** : 33—44.

1953

With Koichi Ishigami
Ecological studies on *Alternaria circinans* causing leaf-spot and browning of cabbage and cauliflower (in Japanese). Bull. Naniwa Univ., Ser. B, **3** : 93—104.

1958

Studies on the pathogenicity of onion downy mildew. Report of survey on downy mildew, Osaka Prefecture, **1** : 1—54.

1959

With Shigeyasu Akai
On the flower-blight of cultivated azaleas caused by *Ovulinia azaliae*. Memoirs Coll. Agr. Kyoto Univ., **80** : 1—20.

Books

1926

An outline of plant protection (in Japanese). 1—408, Yokendo, Tokyo.

1935

An outline of plant disease science (in Japanese). 1—285, Iwanami-Shoten, Tokyo.

1937

Lecture on crop plant diseases (in Japanese). 1—244, Nihon-Noson-Kyokai, Tokyo.

1940

Some problems on plant pathology (in Japanese). 1—479, Nishigahara-Kankokai, Tokyo.

1945

Mycology relating to wood-decay (in Japanese). 1—496, Asakura-Shoten, Tokyo.

1947

Cereal rust and rice blast disease (in Japanese). 1—195, Asakura-Shoten, Tokyo.

1949

Studies on rice blast disease (in Japanese). 1—347, Asakura-Shoten, Tokyo.

1953

New lecture on crop plant diseases (in Japanese). 1—218, Yokendo, Tokyo.

LIBRI NOVI

Pathology. By PETER A. HERBUT, M. D., Professor of Pathology, Jefferson Medical College, Philadelphia. 2nd edition. Pp. 1516; 758 illustrations. Lea and Febiger, Philadelphia, 1959. Price $18.50.

In the preparation of the second edition of this textbook Professor HERBUT was assisted by 21 pathologists and clinicians. The first portions are devoted to the autopsy and to the various types of pathologic alterations. The remainder is organized regionally. A useful addition in this edition is a short section on pathologic physiology at the beginning of each chapter. A fairly adequate bibliography is given at the end of each chapter. Unfortunately, very few references from the last five years are cited.

The book suffers seriously from inadequate editing, even though HERBUT states in the preface that he "has endeavored to correct some of the defects in the first edition" and that "this, of necessity, has added 288 pages and 213 photographs to the book . . . ." There can never be complete agreement as to what is of insufficient importance to warrant inclusion in a general text. However, errors presumably of typography, inconsistencies, perhaps due to the multiple authorship but worthy of explanation, repetition of obsolete and of even bizzarre ideas occur throughout the text. An example of the first is: "Vitamin A is essential to the health and growth of all *vertebrae*", when presumably *vertebrates* is intended (p. 92). In reference to disseminated lupus erythematosus it is stated on page 314 that "Death is usually due to renal failure, cardiac insufficiency, or pneumonia." However, on page 1067 we find" . . . generally the urine is normal and renal insufficiency does not exist." The statement that tinea pedis is "transmitted by bath mats and the like" has been amply disproved (p. 1338). That exfoliative dermatitis "may result from toxemia, gout, tuberculosis, alcoholism . . . ." is without a shred of substantiation (p. 1344).

Mycology is treated in about twelve pages to be found in several portions of the text. The coverage is comparable to that in other general texts.

The defects of this volume detract from its merits to an extent that it must be considered inferior to other current general textbooks of pathology.

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THOMAS G. BENEDEK

Aspergillose der Hornhaut. I. Kasuistischer und experimenteller Beitrag zur Diagnose und Therapie, von G. SAUBERMANN und H. J. SCHOLER. II. Diskussion von 90 Fällen der Literature, von H. J. SCHOLER und G. SAUBERMANN. S. Karger, Basel — New York, 1959. IV—58pp. 9 figs. sFr. 8.— (Bibliotheca Ophthalmologica Fasc. 54)

Contributions of interest to medical mycologists are often scattered, if not hidden, in publications of other disciplines. Therefore, it is necessary and useful to bring them to their closer attention. On the basis of 90 cases from the world literature, the etiology, clinical forms, course and prognosis of corneal aspergillosis are described. As diagnostic aids the authors recommend the cultivation of the pathogenic agent (usually *Aspergillus fumigatus*) and microscopic demonstration of fungus elements from the diseased cornea.

It is a useful publication which may give welcome guidance in this very special field to medical mycologists.

TIBOR BENEDEK

Current problems in allergy and immunology. Papers dedicated to BÉLA SCHICK on the occasion of his 80th birthday. Edited by WILLIAM KAUFMAN. Pp. 992. S. Karger, Basel and New York, 1959. Sfr. 60.—

This Festschrift has been assembled in honor of Dr. BÉLA SCHICK, a pioneer in the study of immunity and an outstanding pediatrician. It is composed of 66 papers, which have also been published in the International Archives of Allergy and Applied Immunology. The majority of the contributions are American, but fifteen countries are represented. Two of the articles are in French, six in German and the remainder in English.

W. B. BEAN provides an eloquent "Homage to Béla Schick." This is followed by two papers of recollections about SCHICK. The other papers present investigations, both fundamental and clinical, in a wide variety of topics, more or less closely related to allergy. It is inevitable that the quality of the reports in such a collection will be uneven. The most interesting papers, because of the promise they carry for further advances are the few which describe investigative technics. One of these concerns the methods for localization of anti-tissue antibodies by labeling antigen with both fluorescein and radioactive iodine (PRESSMAN, D., YAGI, Y. & HIRAMOTO, R., Int. Arch. Allergy, 12:125, 1958).

The clinical papers present little new material. The only one which relates directly to mycology reviews the association of exposure of children to airborn molds and the occurrence of allergic respiratory symptoms (PRICE, H. K. & MORROW, M. B., Int. Arch. Allergy, 15:122, 1959). They discuss the etiologic significance of *Alternaria* and other genera of molds, their climatic distribution, methods of skin testing and of desensitization therapy.

This reviewer is left with the impression that in at least one respect this volume must be disappointing to Dr. SCHICK. For it shows how little fundamental progress has been achieved in the half century since he and his associates made the discoveries which launched scientific immunology.

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DUPERRAT, B.: Précis de Dermatologie. 1106 pp. Extensively illustrated. Masson & Co. 120, Bd. Saint-Germain, Paris (VIe), 1959.

This comprehensive volume is primarily written for purposes of dermatologists. However, it was considered useful to call the attention of medical mycologists, particularly of those of the French language, to this work containing excellent chapters on mycoses. It is useful for medical mycologists when they have a basic insight into the clinical forms of superficial and deep (systemic) mycoses, into the intricacies of their course and get a glimpse at the current therapeutic approach. The author deals systematically with the superficial and deep mycoses giving a concise, clear clinical description, discussing the causative agents and the appropriate therapy which is mainly that of the Hôpital Saint-Louis. The subject matter is critically presented in every respect which makes the text all the more useful. The detailed Table of Contents and particularly the thorough index make it easy to find quickly what the reader desires. The production of the book is impeccable.

TIBOR BENEDEK

ALBERT JAN KLUYVER, HIS LIFE AND WORK, edited by A. F. KAMP, J. W. M. LA RIVIÈRE and W. VERHOEVEN. North-Holland Publishing Co. Amsterdam, P.O. Box 130 and Interscience Publishers, Inc. New York, 1959. Price $ 10.60

To read about the life and work of great men of science has a great inspiring and didactic value. This biography of KLUYVER has been prepared after his death. The biographical section was written by several authors, each one covering a particular period of his life. This section closes with two orations spoken at the cremation ceremonies at Westerveld, the one delivered by the Rector Magnificus of the Technological University and the other one, a testimonial of KLUYVER'S closest friend. The second part of this book brings his "Selected papers" on chemical, biochemical and microbiologal subjects. In the third part there is included his bibliography from 1911 until 1956. KLUYVER'S profound knowledge is apparent in his original papers on microbiological subjects here reprinted. From his own writings KLUYVER emerges as a great scientist. Those who are interested in biography as a literary art and as a form of hero worship will read this book with great interest and enjoyment.

TIBOR BENEDEK

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THE CONCEPT OF SPECIES FROM SUPERIOR TO INFERIOR PLANTS(1)

by

R. Ciferri

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(11.II.1960)

The burden I am shouldering with the subject I am presently to develop is heavy because of three main reasons, in detail : i) Much has been written on the species in general and in particular, but very little has been said of it in reference to Protista; ii) The time I can give to discussion is short, and so I will be forced to resume failing to document with care what I am to affirm; iii) Finally, I am rather less than sure seeing the matter quite clearly now that I sit down to write it.

I should also not fail to remark that the subject which I am to try to cover, would in years past have called for a greater amount of attention than it does today. So much ink has been spilled by now that, if far as yet from ended, still the arguments now smouldering have lost much of their edge. It looks indeed as though we have accepted the idea that taxonomy and systematics are bound to endure in a state of chaos much as the International Rules of Nomenclature have attempted to make matters better, at least in the purely nomenclatural regard. Successful to a point, these Rules have taken care of the formal aspects of the subject without reaching truly deeper, much as some might think to the contrary. Finally, I incline to believe that the current confusion is the byproduct of our increase in knowledge concerning living organisms. As we know more of the individual, so it becomes more difficult to find for every character a fitting pigeonhole on account of interlocking, complex plays.

When a few score of years back the science of genetics came to the fore, and we began to form an appreciation of experimental ecology, hopes soared high that sciences of the kind — as yet virgin, therefore rich of possibilities — would once for all tell us how the species is best to be defined, at the result of informing us concerning the very mechanism of speciation. It so happens, however, that these two sciences have achieved the contrary on account of genetics stressing the genus (or, more seldom, taxa of supraspecific or subgeneric circumscription), while ecology emphasizes taxa of the superspecific (or subgeneric) order, also infraspecific groups from the subspecies downwards.

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(1) Paper read at the Symposium of "Species in Bacteria", International Society of Microbiology, Italian Section, Varenna, 14—15 May. 1958.

Difficulty arises the very moment we attempt to define the species. I will not spend here time in attempting to catalog what different authors have concluded on the score. I will but refer to what MAYR concluded half-jocosely, and everybody is ready to accept with him, that is: "The species is what an informed taxonomist and systematists believes to be a species". It seems to me that our time will be better spent if we discuss, be it so briefly, the origin of the concept of species, which can already be found in the works of ARISTOTLE, but following different attempts at formulation by RAY and TOURNEFORT, reached finally a definite statement at the hand of LINNAEUS. Although the ideas of the illustrous Swede did fluctuate somewhat on the subject, he finally felt sure that the species do exist such as God made them, their limits being fixed in time. It must be admitted that LINNAEUS was rather less than explicit, his position calling for a great deal of controversy, and this might be dangerous to put the matter in the very words I use. Whatever the case in much detail, the Linnean position did mark a step ahead. The old idea still lingered in his times that vegetal species were liable to 'transmutation', whether of microscopic forms (such as were then known only to a very limited extent) or of higher kind so particularly under cultivation. The works of REDI and SPALLANZANI had already demonstrated before LINNAEUS' times that animals and plants were not the subject of spontaneous generation or 'transmutation', but if by now finally rejected concerning animals, still the old idea retained some of its force and appeal concerning plants. Against errors of the kind, the position taken by LINNAEUS could only by welcome.

The Linnaean concept of species endured unchanged for about a century until, that is, the great Darwinian turn. It seems worthy of note that while LINNAEUS had particularly busied himself with the systematics of plants in the wild without for this failing to pay a measure of attention to cultivated stock, whether plants or animals, DARWIN's thought was essentially polarized in the direction of plants and animals under cultivation and domestication. Accordingly, DARWIN dealt with a material beyond comparison more plastic than the one in the hands of LINNAEUS, material which would mutate right on the spot and then last through centuries. In the light of his own experience, DARWIN of course reached conclusions opposite to LINNAEUS'. Without denying the existence of species, the great Britisher affirmed in no uncertain terms the fact that they were liable to change and alteration. Toward the end of his life, he loned down his position somewhat, granting by then that the species is variable, but that it "stays put" for a long period of time nevertheless.

The Darwinian position started arguments which lasted for half a century but stemmed, as we now well know, from a fundamentally erroneous premise. LINNAEUS had associated, it is true, a concept of species with a concept of fixity of species, but this association would not necessarily mean that the two concepts are inextricable. In other words, the fact that the species is liable to vary, does not mean that the species does not exist. Whatever the case, the arguments that thus arose split the learned world into two opposing fields, a tendency to deny the existence of species being all the more marked in the ranks of naturalists who dealt with life lower in the scales of evolution.

We should here think of the fact that, in spite of everything, a general idea of evolution has constantly gained ground following DARWIN'S time, so much that, as BERTALANFFY claimed, this idea has by now the status of a true postulate in logics. This idea, let us remark, runs against the concept of species, because if evolution actually takes place, the species pass one into the others, which but can mean that the species then ceases to exist. This idea has led some to accept a doctrine of transformism, but this is a question with which I cannot be concerned here.

Whatever the case, today everybody agrees that, whether the species has genuine existence in nature, or it but represents an abstraction, it stands for a category which we cannot avoid, using, even though it forces us to describe as static, what is in reality quite changeable.

DARWIN'S ideas brought about corollaries the importance of which was not felt on the spot. So long as everybody stood by the Linnean concept of species a problem in descent did not arise, but with DARWIN the way was open to enquiries into phylogeny, that is, into attempts at building a tree-of-life in relations to two coordinates in space and to one in time. Speaking with LINNAEUS, the naturalist could only refer to a resemblance between species according to the Creator's own will, but with DARWIN the door was open to discuss issues of consanguinity.

In addition, the advantage of darwinism is the "economy" of the theory, the scientific spirit introduced in the evolutionary matter. But DARWIN himself recognized that, emphasizing the classical aspect of the natural selection, it does not explain the essential of the problem: the origin and ramdomness of the variations, as related with the order of apparition and the coordination - the functionality - of the modified structures. Species and genera are scarcely distinguishable on the base of adaptative criteria, and not at all the superior taxa (families, orders). Even in the most modern aspect, darwinism is to be accepted "faute de mieux".

Generally speaking, we understand today the species in immediate reference to two different sets of criteria, as follows : i) Morphological, by which is mainly stressed the interrelationship of resemblance or dissimilarity; ii) Genetic, based on the fact that gene flow is only intraspecific. A third criterium exists, which is ecogeographic, but this merges, I believe, with the genetic one. I here stress a *criterium* rather than a *concept* of species, because I believe that a *conceptual species* is imaginary. What has real existence are *different kinds of species* definable in different living groups to agree with the ideas of the specialists concerned with their classification. In addition, of course, the species may be assumed as a concept in the abstract of essentially philosophical and speculative nature, or purely as a category serving immediately practical needs, and so essentially utilitarian.

Summing up, I would be inclined to state that the criterium of species, as I might call it, rests upon a kind of judgement, as it were, which we formulate in regard of a collectivity of living beings compared with similar collectivities, in order to soar finally from details on to general.

The difficulty of individualizing the species, already serious in the regard of certain groups of higher animals and plants, has become by far more serious when the naturalist was brought increasingly to face beings low down in the scales of life. Indeed, naturalists have been inclined to generalize the concept of species in application to beings of the kind without stopping to enquire whether it was consistent to generalize this concept in their immediate regard. Practical difficulties soon arose in consideration of the scarce morphological variability met at this level. Animals and plants of the lower kind answer a few simple morphological patterns, and hardly offer variations of marked importance. The circumstance proved none the less unfortunate that morphologically, and later also biologically, assumed species were given currencies for life of the kind, long before its students could form a proper idea of its range of variability.

A new difficulty was added to the old ones when it dawned upon the students in question that large groups of microorganisms had no sexual cycle, therefore could not be made the subject of genetic enquiry to determine their specific rank. This difficulty was, of course, in part turned by sheer taxonomic acumen, and it must be granted that acumen of the kind did prove a marvellous instrument of effective disposal. Striking is indeed the record of the early systematists and taxonomists in the premises.

The difficulty I have just mentioned is by no means the last one in score. Microorganisms are as a rule exceedingly plastic and very widely distributed, which means that ecogeographic criteria of specific discrimination cannot be introduced in their regard.

The criteria as to what makes a species in different living groups do vary in time because in the very measure that time elapses we know more in depth, and it has by now become very difficult to understand what is meant by the classification of the early authors in protistology. There is a definite historical tendency for all the taxa to gain in rank . Frequent is the case when old species become genera turn into families, and families into orders. At the same time, the number of taxa, intermediate between species and genera, genera and families, families and orders, is constantly on the increase. Overall, it is not imprudent to conclude that there is a clear tendency at one hand in the direction of a finer systematic subdivision of living beings, at the other hand in the direction of a multiplication of intermediate taxa, which but means an open recognition of the gradual nature of the passage among different groups. As this work goes on, we are by degrees awakening to the realization that the differences which distinguish one taxon from the other are markes not only by affinities and differences, but in addition by a certain "something" about which we are not quite sure at this hour.

Today too, systematists tend to break apart as "splitters" and "lumpers". Using the very same material the two schools disagree. The lumpers stress resemblances, the splitters differences, and what results is rather more a matter of temperament than a genuine preoccupation with the objective background of the facts. Naturally, it seems useless to mention here in addition difficulties which arise from technicalities of formal nomenclature. To these difficulties the International Rules try to put some remedy, but so only in the spirit of BRIQUET, who admitted that if the Rules in question are uncertain, let them be at least "consciously" uncertain.

Fortunately for science, *biosystematic concepts* have been developed, which shift the center of interest away from the individual toward the population, understanding the latter as a sum of living beings forming something of its own, not unlike a wall stands for something more than a simple pile of bricks. These concepts have stressed the physiology of the collectivity which cannot be only that of the single individual, no more so indeed than the function of a tissue is only that of the cells which make it up. Granting that from the biological viewpoint, the population has individuality, physiology and morphology of unitary nature, it follows that we can only understand the species in terms of the genetics of the collectivity, that is, of the pool of genes it has in common. This approach has been made all the more easy in that we have been forced to work on microorganisms in cultures, that is, at the level of populations, and we have thus learned of factors of pathogeny and symbiosis which we could not learn when counting on the lone individual.

In relation to the above, we have shifted the concept of species from higher beings with sexual reproduction to lower ones, including forms having asexual reproduction, to match clonal populations of the kind which in higher apomictic plants, DU RIETZ has identified as *agamospecies*, without for this stressing the genetic aspects of the variability and its manner of formation, such as are necessarily different in higher and lower beings. Variability by hybridisation and allopoliploidy have of course no longer place in the latter ones. Autopolyploidy, if present, is rare and may have a different meaning than in the higher plants. Genetic variability is of course left including the possibility of mutation, and certain mechanisms as, e.g., heterocaryosis, on the score of which we are as yet poorly informed. Average mutability and a greater plasticity tend to compensate for the above limitations, and may indeed influence variability in general.

The clones of populations which are self-fertile or apogamic, or better yet to say, necessarily bound to vegetative reproduction, are systems closed and isolated on account of non transference of genes. Genotypical analysis accordingly being very difficult when not impossible altogether, and the species being accordingly reduced to biotypes that are similar and relatively constant, the "rank" of differential characters becomes entirely arbitrary, and it appears altogether likely that selection acts on life of the kind in a manner different from the one current in regard of forms having sexual reproduction. In the end, the conclusion seems proper that the species understood as a static sector of the evolutive *continuum* of living organisms, loses its meaning altogether in regard of Protists.

More ambiguous and nebulous still became the task of identifying the species when the Eucariotes were left behind thus to reach the Procariotes, that is to say organisms wanting a nucleus of the kind standard for plants and animals. It is right unfortunate that safe criteria of identification are needed precisely in connection with beings exhibiting a nucleoma rather than a nucleus, beginning with the Schizomycetes. Life of the sort has major interest in the sense of pathogeny, technology and therapeutics, and its mechanism of variability (as, e.g., cytoplasmatic inheritance) may mean something quite different than it does in higher organisms.

We are accustomed to construe morphological manifestations in the sense, so to speak, of a tangible expression of bioecological facts, but this proves impossible, unless it be in connection with certain broad taxa, among microorganism, and Procariotes in particular. The morphology of life of the kind is of the rudimentary kind, and convergence among forms derived from different phyla is so prevalent that specific definition can only be sought in biological and biochemical characters, the latter most particularly, and be formulated on the strength of physical and biophysical properties.

The following tabulation is an attempt to list the criteria of specific discrination in microorganisms:

A) Morphological characters

  a) *Macromorphological characters* established upon morphological differences of outstanding significance and of easy appreciation.

  b) *Micromorphological characters* drawn from less important morphological differences and particularities.

  c) *Biometrical characters*

B) Biological characters

- a) *Characters of specialization "in vivo"*, in connection with parasitism.
  - i) *Characters of specialization of matricial order* (a microorganism can live only on certain hosts, but not on others, or only upon a given living substratum).
  - ii) *Characters of organotropical organization* (a microorganism can live only upon certain organs or groups of organs of the host).
  - iii) *Characters of pathognomony* (the effects of the microorganism upon the host, and the latter's reaction, as for example symptoms and decurrence of the infection in the case of its being of parasitic nature, reactions of anatomo-histopatological and biochemical kind, modifications in the living substratum in the case of saprobiosis, etc.).
- b) *Characters of specialization "in vitro"*, in the case of life of the saprophytic order, particularly under culture.
  - i) *Physionomic characters* (colour, shape, texture, rapidity of growth, etc.).
  - ii) *Physico-chemical characters* (for instance, thermal point, resistance and modifications of the pH, modifications in the conditions and viscousness of the substratum, resistance to unfavourable agencies, etc.).

C) Biochemical characters (i.e., products of anabolic or catabolic activities, etc.).

Species have been described (particularly among Fungi and Bacteria) on the basis of one or more of the characters above stated, and if validly published, there is no reason why they should not be accepted. However, the question, indeed, remains what is meant by the term "species" when this term is applied to a heterogeneous assemblage of forms which, though all labelled as "species", cannot be compared one with the others, and are identified on the strength of different criteria of determination. As MAYR said, nothing here remains but a vague rank of "species".

It seems clear that this state of confusion which associates as one, individuals and collectivities, parts and wholes, objective and subjective data, is necessarily unwelcome not only from the formal standpoint (that is to say, in relation with taxonomic and nomenclatural considerations) but also in the more important respect of basic research, the ultimate purpose of which can only be the identification of living groups as such, and in its practical applications.

Matters are likely to become worse because it is probable that more advanced knowledge will make it possible to identify more "species" in the future. It has been stated that if, for instance, we investigate the biochemism of living organisms on the basis of 18 aminoacids, 6 pyrimidines, 12 vitamines, 6 monosaccharids and 12 mineral elements, we are bound to reach a total of 10^{130} possible combinations which can only mean, I should think, that we shall end by considering each individual as a "species".

This is not all, by any means, because two other factors are bound to intervene which will increase confusion. One of these factors is to be found in our typological approach, the other in the manner and purpose of specific arrangement.

Concerning the former, we cannot fail to apply a concept of typology which bears little relation to the one now current indeed. It is clearly absurd to base the species in the Protists on the population only next after to refer it to a type which may not — and often does not — represent the type of the species as a collective entity. It is by no means easy to pass from the concept of type-individual to that of type-culture, if we only consider that the cultures are variable and subject to degradation by repeated transference.

Attempts made at identifying the type-specimen as the "label-bearer" (onomatophore) of the species cannot rescue us from the ambiguity of designating a type, which represents the species without its standing as an average individual. It seems to me better to retain the old abstract concept, formerly identified as "typicus", "communis", "genuinus", or the ideal average type.

In the following tabulations certain basic differential characters are offered to discriminate animals, plants and Protists (especially Protophyta) characters of a kind that immediately interest the specific concept in different groups:

ZOOLOGICAL SPECIES	BOTANICAL SPECIES	
	In the higher plants	*In the lower plants*
POLYPLOIDY		
Not frequent	Frequent	Rare
ASEXUAL REPRODUCTION		
Rare	Frequent	Current
INTERSPECIFIC HYBRIDIZATION		
Rare	Fairly frequent	Very rare
HETEROCARYOSIS		
Virtually none	Rare	Current
MUTABILITY		
Rare	Fairly frequent	Frequent

	BIOCHEMISM	
Homogeneous and uniforme	Fairly well differentiated	Highly different.
	PLASTICITY	
Scarce	Fair	Very high
	SPECIES MAKING AT THIS HOUR	
Sluggish	Moderately active	Very active

It is my understanding that whatever we have discussed up to this point will be clearer, perhaps, if we turn our attention to questions that immediately concern classification in general.

In the English language taxonomy is synonymous of systematics which is not what a Latin-speaking author can accept.

De Candolle (1813) discriminate *taxonomy* from the *systematics* of Linnaeus (1753) intending as the former the study of ranks (taxa) effected in relation with the characters chosen for systematic and classificatory purposes. It will be noted that *taxonomy* and *systematics* are not synonymous as it is currently believed, but only in part correspond to what Mayr *et al.* have understood as *alpha* and *beta* taxonomy.

The student of nature forever attempts to bring all living organism — be it only group by group — within the frames of a primordial scheme which considers every one of the possible degrees of affinity, past or present. This attempt has for its result to establish *systematics* of the *natural* kind, by which life is allotted to different phylogenetic trees implying descent and evolution through time.

Once this work is done, the naturalist who intends to use it for purposes of immediate determination, arranges the different characters in orderly series of groups which are currently understood as *keys*. These *keys* do not take into account everyone of the characters implying either similarity or dissimilarity, but concentrate only upon certain characters of easier identification. *Classification* which is not at the same time *systematics*, obviously stands as but a partial and artificial arrangement, or at the very best only as a first step toward *systematics*.

To exemplify in the most direct way possible: We can *classify* the books in a library according to certain external characters, as to size, bulk, colour of the binding, etc. An arrangement of the kind may be convenient, but does not arrange matters by order of their affinity. If, by contrast, we classify the books in question in relation to their contents, what results, is of course a true *systematic* arrangement. Naturally by *systematizing* thus the books in question we do not deprive ourselves of the possibility of additionally taking into account characters of classifications as above. To tag a book as a mark of easy recognition answers what in the natural science is the naming of a taxon.

It seems accordingy clear that it is a mistake to confuse classification *a posteriori* (such as is derived from systematics, and may eventually be simplified into a key) with classification *a priori*. It is my definite understanding that in the last few years all our work has been purely *classificatory* much as many have believed the contrary and not genuinely *systematic*. It is only through *biosystematics* that we may enter the field of *genuine systematics*. In a sense, present conditions are understandable as a preliminary step in knowledge but not beyond.

At bottom, the difference between *systematics* (or, as we might perhaps call it, *natural classification*) and *artificial classification* is to be found in the number and quality of the characters considered, that is, in the amount of interrelationships established and accepted. The species, assumed at the level of praxis, is a matter of classification and only becomes a subject of systematics under a higher level of handling. In terms of genetics, I believe that the work to come may be conducted to answer the viewpoint of SHULL, for whom each living organism has a core of genes that are unchanged in relation to the species, and genes on the contrary which are variable in the individual (and, as I might add, in the population). It is my feeling that the species should be based on the rockbottom of genes which are invariable, while genes that are still liable to fluctuation may properly be used to bolster infraspecific taxa, always taking, of course, into account phenotypic variations answering ambiental conditions. We may well find useful in this connection the homeostasis of LERNER, covering lines development which tend to become fixed in time.

Time is now to conclude. In my opinion, we cannot seek a remedy to the current state of affairs having recourse only to conventional devices, because if we do the genuine extent of our knowledge is not bound to gain. Man's activity is surely rather less than orderly when we think of exploring other planets without being in the condition to know well our own.

My main concern is, of course, with microorganisms, and those in particular which do not have sexual reproduction. In submitting the considerations immediately next to follow, I do not pretend of course hopefully to codify the matter, only to lay down a constructive line of endeavour for the future, thus:

1) In microorganism with vegetative reproduction we may consider as species a series of analogous populations which are separated from clearcut gaps. We may assign to these series a conventional binomial in Latin form. Clonal populations under culture should not be separately identified. He who happens to have long familiarity with microorganisms is readily induced to believe that the species

in life of the kind is represented by linear series, the extremes of which are of easy identification but connected by endless intergrades. The different components of this series should be understood as clones to be individualized by figures or letter. As an alternative, we might adopt a trinomial nomenclature, the varietal term of which may designate an individual population to the series. In classifying Penicillia or Aspergilla we may stop with the series — which I view as the true species — without accepting the concept of "species" advanced by THOM and RAPER, who understand as species what are to me but clones, etc. The same applies to aerobic Actinomycetes, the "new species" of which generally answer sheer commercial preoccupations without bearing on systematics, perhaps not even on classification.

2) The species (or series) should be recognizable by several different characters (not all of them necessarily biochemical), having regard to the essential fact that in nature species-making would seem to be effected by modifications that are mutiple, parallel and simultaneous. Individualized by only a few characters, a group can hardly be better than a clone. It is currently accepted in systematics that a group recognizable by only a minimum of characters cannot have rank above form (ROTHMALER), and must in any case remain at the infraspecific level.

3) It is desirable that when identifying a group mention should be made: a) of its main differential characters; b) how these characters stand subordinated one to the other. Specialists in different groups should undertake continuous revisions of the criteria of classification that may be desirable, thus to conform with the technical and general progress of knowledge. It is obvious that the characters of specific identification do vary from group to group but, though obvious, the fact is not always properly taken into account. To illustrate: characters which may be valid to set aside species in Ferrobacteria are of no value in relation, e.g., to Salmonellae, the same being true of Actinomycetes and Thiobacteria etc.

Broadly speaking, the criteria of discrimination for orders and higher taxa are the very same valid from families to genera, but their hierarchy (order of importance) may be liable to opportune modification.

The identification of a clone should not be restricted, but that of a species should be reserved for a specialist in the particular group who alone can have adequate appreciation of the limits of basic variability. The point I thus underscore is beyond question quite important. If, for instance, we take a few Actinomycetes, and divide them into a score of population, the chance is great that we will eventually find among them"species" which seem to be isolated and so quite well defined. However, if some two thousands populations are studied as one, "species" of the kind readily turn out to be connected by intermediates. In other words, the larger the material, the less the probability of finding in it genuine species.

My parting statement is that I am far from having covered the subject as it ought to be, nor am I sure always having been very clear. I hope nevertheless that these notes may contribute to the progress of the science in which we jointly labour. (1)

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(1) My warmest thanks are due to my friend Dr. L. CROIZAT, Italo-american botanist, for the translation from Italian to English language.

THE INCIDENCE OF AIR-BORNE FUNGUS IN SYDNEY

by

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F. R. A. C. P., M. C. P. A.

In view of the widespread occurrence and clinical significance of mould allergy, it is of considerable importance to accumulate data on the number and kinds of fungi in the air at different seasons of the year. The history of work in this field is well described by FEINBERG[1]. Apart from being helpful to the allergist, such data permit an interesting comparison with similar data compiled in other countries. The aim of this study was to obtain information on the kinds and prevalence of moulds of the air in Sydney and to correlate their seasonal fluctuation with meteorological data.

METHODS

This work covered a whole year, from March 1, 1958 to February 28, 1959. The culture plate technique, although of admittedly low trapping efficiency, was used because of its simplicity and because a large body of comparative data from other parts of the world is available. It is hoped to continue this work in the future using an air-sampling device. Two standard 100 millimetre Petri plates containing Sabouraud's agar adjusted to a pH of 5.6 were exposed for ten minutes each day at 3 p.m. for five days every week. For the site of exposure a relatively unobstructed window-sill was chosen on the seventh floor of the nurses' quarters in the vicinity of our Institute. After exposure the plates were sealed and incubated at room temperature for five to seven days, after which the colonies that developed were identified and recorded. The plates were subsequently re-examined for the late appearance of slow growing colonies. Colonies producing no spores on the first occasion were re-examined at a later date, and those which after repeated examination did not produce spores were labelled Mycelia sterilia. Genus identification alone was undertaken. Unidentified colonies were kept for further study.

RESULTS

There were 520 Petri dishes exposed during the whole year. Table I lists the various genera in order of their frequency and the total

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*) Working with the help of a full time grant from the National Health and Medical Research Council of Australia.

TABLE I.

Plate Counts of Air-borne Fungi

Genus	1958										1959			% of
	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Total	Total
Cladosporium	51	55	71	70	60	70	80	61	61	86	42	39	746	19.2
Alternaria	33	44	13	42	17	24	42	46	55	89	128	132	665	17.1
Epicoccum	6	23	31	68	43	35	54	56	94	92	53	54	609	15.6
Pullularia	3	7	16	12	40	26	16	39	20	32	30	18	259	6.6
Penicillium	13	17	18	17	17	18	34	28	19	31	15	20	247	6.1
Phoma	21	6	1	3	6	6	9	15	2	9	6	3	87	2.2
Helminthosporium	12	18	1	1	—	3	4	4	5	4	10	20	82	2.1
Streptomyces	13	8	1	2	3	1	—	6	7	6	11	7	65	1.7
Fusarium	1	1	1	1	11	5	3	13	6	6	6	8	62	1.6
Cryptococcus (red-orange)	2	7	1	6	—	6	1	3	2	—	31	3	62	1.6
Stemphylium	4	5	6	1	—	—	—	3	9	6	2	20	56	1.4
Acrothecium	—	—	—	—	10	—	3	11	7	5	6	7	49	1.3
Aspergillus	12	9	2	—	—	4	3	2	4	3	2	3	44	1.2
Nigrospora	4	5	2	3	—	1	2	—	1	1	8	15	42	1.1
Oospora	16	9	5	4	1	3	1	—	—	1	2	—	42	1.1
Pleospora	—	1	6	3	1	1	1	1	2	7	7	8	38	1.0
Cephalosporium	6	3	5	1	—	1	1	1	1	3	4	4	30	0.8

Sepedonium	—	—	—	—	—	1	—	4	5	—	—	—	10	0.3
Botrytis	—	—	—	—	—	—	—	2	1	—	3	3	9	0.2
Trichothecium	—	—	—	—	2	2	1	—	—	—	—	1	6	0.2
Monilia Sitophila	—	—	—	—	—	—	1	—	1	—	3	—	5	0.1
Cunninghamella	—	—	—	—	—	—	—	—	4	1	—	—	5	0.1
Scopulariopsis	—	3	—	—	—	1	—	—	—	—	1	—	5	0.1
Syncephalastrum	—	—	—	2	—	1	—	—	—	—	—	—	3	0.1
Torula	—	1	2	—	—	—	—	—	—	—	—	—	3	0.1
Trichosporon	—	—	—	—	—	—	—	—	—	—	1	1	2	0.1
Mycelia sterilia (white)	44	5	28	21	22	41	26	29	24	36	13	4	293	7.5
Mycelia sterilia (dark)	29	16	9	—	1	—	—	10	11	8	11	12	107	2.7
Unidentified	9	15	11	6	12	3	8	16	22	10	15	26	153	4.0
TOTAL COUNT	293	268	235	269	252	254	294	365	378	447	417	421	3893	100%
Average temp. (Deg.) F.	72.3	67.3	64.8	57.3	54.7	57.7	57.9	64.5	70.1	69.5	72.5	72.9		
Number of rainy days	17	14	7	15	6	9	11	11	12	17	16	11		
Average Relative humidity	63%	55%	47%	62%	43%	49%	53%	51%	57%	60%	66%	66%		
Prevailing wind direction	NE-E 36% W 27%	NE-E 37% W 26%	W 60%	W 46%	W 63%	W 57%	W 39%	W 41%	NE-E 54%	E-SE 39%	NE-E 44%	NE-E 56%		

number of colonies per month of each genus. A total of 3,893 colonies were counted, of which 153 (4.0%) were not identified. December, February and January recorded the greatest number of colonies with December at the peak. It can be seen that *Cladosporium* was the fungus most frequently encountered throughout the whole year, forming 19.2% of all colonies. During eight months of the year, from March to October, *Cladosporium* was the predominant fungus. *Alternaria* was second in frequency followed by *Epicoccum*. *Alternaria* was the fungus most commonly encountered during January and February, and *Epicoccum* during November and December. *Cladosporium*, *Alternaria* and *Epicoccum* combined comprise 51.9% of all colonies.

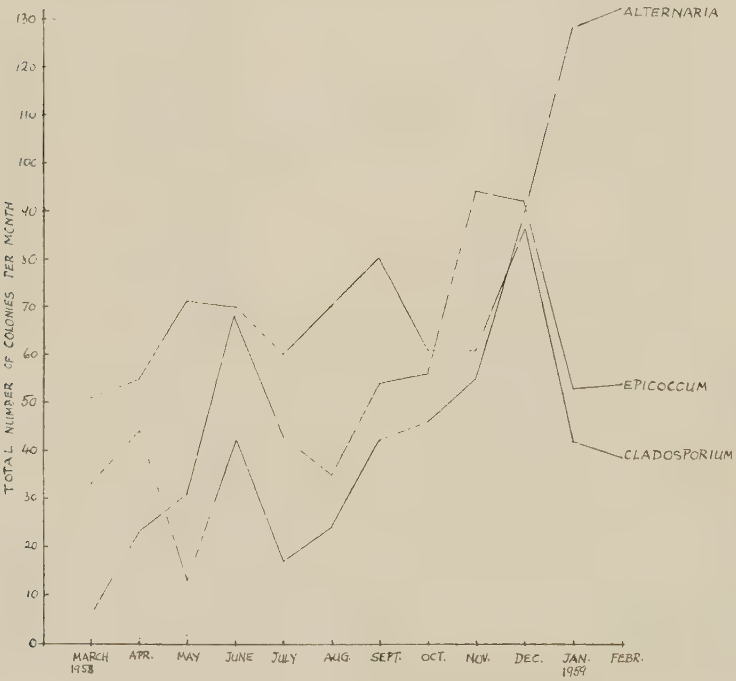

Fig. 1. The seasonal variation of Alternaria, Cladosporium and Epicoccum.

Table I also shows the monthly variations in average daily temperature, average relative humidity, number of rainy days, and the prevailing wind directions. Fig. 1 shows the monthly change in level of incidence of *Cladosporium*, *Alternaria* and *Epicoccum*. It will be noted that the curve representing *Cladosporium* attains three peaks, the first one is the lowest, extending from May to June, the second peak is in September, and the third and highest is in December. Similarly, *Alternaria* exhibits three peaks of incidence during the year, one in April, one in June and another in February. *Epicoccum* has two peaks, one in June, another in November. The high summer peak of *Alternaria* is noteworthy in view of the falling levels of incidence of both *Cladosporium* and *Epicoccum*.

Fig. 2 shows a comparison of the average curve of incidence of the total fungus population with three graphs representing average daily mean temperature, relative humidity and the number of rainy days. There is a rough correlation between these three curves. The slight decline of the average curve of mould incidence corresponds to the fall of all three meteorological graphs to a point in May. From this point on, while the average daily temperature continues to fall, the average curve of the total fungus population together with those of relative humidity and rainy days exhibits a rise to a peak in June. A recession from this peak occurs to a low point in July, then a general rise of all curves takes place to another peak in December.

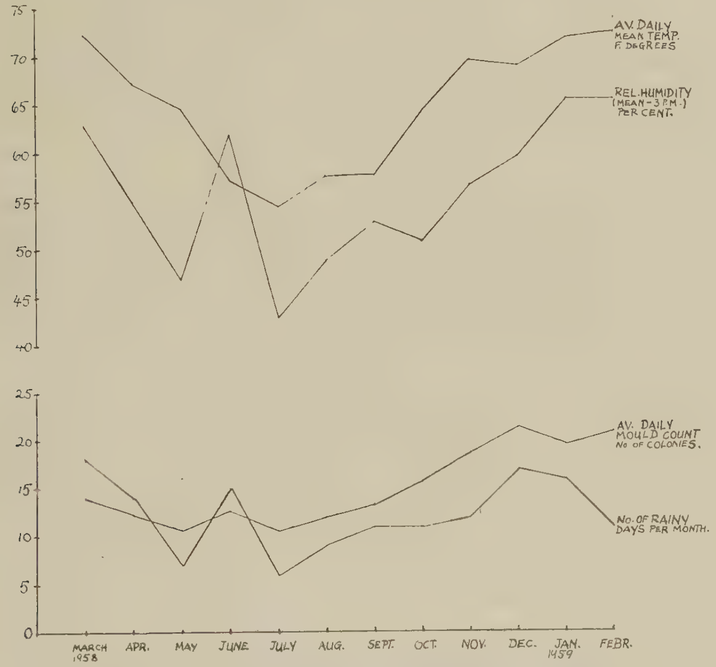

Fig. 2. Comparative graphs of Av. Daily mould count and meteorological data

DISCUSSION

The predominance of *Cladosporium* spores in the locality of Sydney is not surprising. *Cladosporium* is ubiquitous and its dominance in other parts of the world is well documented. It has been found the prevailing air-borne fungus in England[2-3], in New Zealand[4-5], and in several areas in U.S.A.[6-7-8]. The seasonal pattern of *Cladosporium* here appears to be different from that which has been reported in England. Hyde & Williams[9] found low *Cladosporium* counts for nine months of the year and relatively high values for the three summer months. A similar high summer and low winter incidence was found for both *Cladosporium* and *Alternaria* in the northern half of the U.S.A.[10]. The pattern of incidence of *Cladosporium* in the Sydney area is different in that it exhibits no very marked fluctuations up to December, and it displays three peaks and a marked fall from its December peak to low values in the summer months January and February. (The winter in Sydney is, of course, much milder than that in England or the northern part of the U.S.A.). The counts of *Alternaria* fall into a pattern which shows an autumn, an early winter and a high summer peak. This three peak pattern appears to bear some resemblance to the findings of Targow & Plunkett[7] who found that apart from an increased incidence of both *Cladosporium* and *Alternaria* during the summer, there were noticeable peaks also in spring and autumn. The occurrence of *Epicoccum* as a predominant organism is of interest. We have found only one other reference of such high numbers occuring in the literature. Dye & Vernon[5] found that it was the second most common mould encountered and made up 12.1% of all the fungi encountered. Although the general consensus of opinion is that the seasonal variations prevalent in air-borne fungi are influenced by climatic factors, apparently it is not always possible to establish a clear correlation between prevalence of fungi and climatic factors.[11]. In this survey the differences in prevalence of fungi correspond roughly with differences in the temperature, relative humidity and rain fall records. The only discrepancy seems to be a rise in fungus incidence and a simultaneous fall in temperature from May on. However, in this instance a marked rise in relative humidity might have offset the effect of the falling temperature.

No clear correlation could be established between colony counts and wind direction and velocity. Observation on the wind direction at 3 p.m. during the summer months (December, January, February showed that the wind was from the ocean about 91% of the time, reckoning the following as ocean winds - E, S, NE, ENE, NNE,ESE, and SSE. It is perhaps unexpected to find that the highest prevalence of fungi occurs when the prevailing wind direction is from the sea. In this connection it is interesting to note the findings of Harsh & Allen[8] of San Diego, California. According to their survey the wind was from the ocean about 90% of the time in San Diego. These authors found that when plates were exposed on a fishing vessel at distances up to 30 miles offshore from San Diego the types and numbers of colonies were similar to those found on the land, even when the wind in each case was from the sea.

Acknowledgments.

It is a pleasure to thank the Director of the Meteorological Bureau, Observatory Park, Sydney, for information regarding the weather.

SUMMARY

A survey of the incidence of air-borne fungi in Sydney was carried out for one year by daily exposure of Petri plates containing Sabouraud's medium. A total of 3893 colonies were counted.

The fungi most commonly grown were *Cladosporium, Alternaria* and *Epicoccum*; these accounted for 51.9% of all colonies.

An attempt was made to correlate the work with the average daily mean temperature, the relative humidity and the number of rainy days. No clear correlation could be established between colony counts and wind direction and velocity.

ZUSAMMENFASSUNG

Eine Untersuchung über das Vorkommen von durch die Luft getragenen Pilzen in Sydney wurde während eines Jahres durch tägliche Ausstellung von Petri-Schalen mit Sabouraud's Nährmedium durchgeführt. Insgesamt sind 3893 Kolonien gezählt worden.

Die am häufigsten gefundenen Pilze waren: *Cladosporium, Alternaria* und *Epicoccum*; sie umfassten 51.9% aller Kolonien.

Ein Versuch wurde unternommen, um diese Befunde mit der durchschnittlichen, täglichen Mitteltemperatur, der relativen Feuchtigkeit und mit der Zahl der Regentage in Korrelation zu bringen. Keine klare Korrelation konnte zwischen Kolonie-Zahl, Windrichtung und Windgeschwindigkeit festgestellt werden.

References

1. Feinberg, S. M.: Allergy in practice, pp. 1—838. Chicago. Allergy to fungi, pp. 216—84 (1947).
2. Hyde, H. A. & Williams, D. A.: A census of mould spores in the atmosphere. Nature, Lond. **164,** 668—9 (1949).
3. Ainsworth, G. C.: The incidence of air-borne Cladosporium spores in the London region. J. gen. Microbiol. **7,** 358—361 (1952).
4. Di Menna, M. E.: A quantitative survey of aerial moulds and yeast in Dunedin, New Zealand. Trans. Brit. Myc. Soc. **38,** 119 (1955).
5. Dye, M. H. & Vernon, T. R.: Air-borne mould spores. A New Zealand survey. N.Z.J. Sci. Tech. B. **34,** 118 (1952).
6. Durham, O. C.: An unusual shower of fungus spores. J. Amer. med. Ass. **111,** 24—5 (1938).
7. Targow, A. M. & Plunkett, O. A.: Incidence of Atmospheric Spores in the Los Angeles Area. Ann. Allergy, **9,** 428—445 (1951).
8. Harsh, G. F. & Allen, S. E.: A study of the fungus contaminants of the air of San Diego and vicinity. J. Allergy, **16,** 125, (1945).
9. Hyde, H. A. & Williams, D. A.: The incidence of Cladosporium herbarum in the outdoor air at Cardiff, 1949—50. Trans. Brit. Myc. Soc. **36,** 260 (1953).
10 Morrow, M. B.: Mould fungi in the etiology of respiratory allergic diseases. VII. Further survey studies Ann. Allergy, **5,** 442 (1947).
11. Lacaz, C. S., Mendez, E., Pinto, R. Y. D., Cardoso, T. H. B., Lucchetti, L. C. H.: Fungos anemofilos mas cidaded de Sao Paulo e Santos (Brasil) Rev. Hosp. Clin. **13,** 187—193 (1958)

ADDITIONS TO FUNGI OF BOMBAY — V.

by

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Since the publication of the list of Bombay Fungi in 1934 by Uppal, Patel & Kamat, several authors have published additions to the list, the last being that of Chiddarwar (1958). The present list deals with the new additions of Ascomycetes and Fungi Imperfecti collected by the authors from the various parts of Bombay State, particularly the Poona district. Species new to science have been deposited in the herbaria of the Commonwealth Mycological Institute, Kew, Surrey, England and the Indian Agricultural Research Institute, New Delhi, India. The genera have been arranged alphabetically under the two classes of fungi. The genera *Cyclotheca, Melomastia, Salmanomyces, Scolecodothis, Microcyclus, Sydowia* and *Tympanopsis* are new records for India.

The writers are grateful to Prof. M. N. Kamat for his encouragement in the preparation of this list and to Dr. S. P. Agharkar for facilities offered at this laboratory. Thanks are also due to Dr. Emil Muller of Swiss Federal Institute of Technology, Zurich and Dr. von Arx of Phytopathological Laboratory, Baarn, Netherlands, for their kind help in the identification of some Ascomycetes.

ASCOMYCETES

1. *Acanthostigma heterochaete* Syd. & Butler.
   On the leaves of *Alysicarpus vaginalis* DC. Poona, July-Aug 1958. Leg. S. T. Tilak.
2. *Anthostomella pandani* Sacc.
   On the leaves of *Pandanus fascicularis* Lam.
   Vithalwadi, Poona, 15th Sept. 1958, Leg. S. T. Tilak.

*3. *Chaetothyrina* sp.
   On leaves of *Chonemorpha fragrans* Alston.
   Khandala, 6th Oct. 1958, Leg. S. T. Tilak.

*4. *Cyclotheca kamatii* Viswanathan.
   On leaves of *Ligustrum nilgherrense* Wt.
   Mahabaleshwar, 1st Feb. 1958, Leg. T. S. Viswanathan.

*5. *Didymella mulleri* Viswanathan.
On dried stems of *Duranta plumeri* Facq.
Poona, 9th Feb. 1959, Leg. T. S. Viswanathan.
*6. *Diaporthe* sp.
On stems of *Cassia tora* L.
Poona, 10th Feb. 1959, Leg. T. S. Viswanathan.
7. *Erysiphe matheranensis* Viswanathan.
On leaves and stems of *Leucas stelligera* Wall.
Matheran, Dec. 1956, Leg. T. S. Viswanathan.
*8. *Leptosphaeria helianthi* Tilak.
On dried stems of *Helianthus annuus* L.
Poona, July, 1959, Leg. S. T. Tilak.
9. *Lophodermium agharkarii* Tilak.
On leaves of *Syzygium cumini* Skeels.
Poona, 20th Jan. 1959, Leg. S. T. Tilak.
10. *Lophodermium indicum* Tilak.
On leaves of *Lagerstroemia flosregina* Retz.
Poona, 15th Jan. 1959, Leg. S. T. Tilak.
*11. *Massarina jasminicola* Viswanathan.
On stems of *Jasminum malabaricum* Wall.
Poona, 9th Feb. 1959, Leg. T. S. Viswanathan.
*12. *Melomastia* sp.
On bark of *Syzygium cumini* (L.) Skeels.
Poona, 10th Feb. 1959, Leg. T. S. Viswanathan.
13. *Microcyclus indicus* Tilak.
On leaves of *Actinodaphnae hookeri* Meissn.
Mahabaleshwar, Feb. 1958, Leg. S. T. Tilak.
14. *Mycosphaerella artemisiae* Tilak.
On leaves of *Artimesia nilgirica* Pamp.
Purandhar Fort, Aug. 1958, Leg. S. T. Tilak.
15. *Mycosphaerella bombycina* Viswanathan.
On leaves of *Syzygium cumini* Skeels.
Poona, 15th July, 1958, Leg. T. S. Viswanathan.
16. *Mycosphaerella calotropidis* Viswanathan.
On leaves of *Calotrpis gigantea* R. Br.
Poona, 12th August, 1958, Leg. T. S. Viswanathan.
17. *Mycosphaerella cassiae* Tilak.
On leaves of *Cassia tora* L.
Pona, 28th July, 1958, Leg. S. T. Tilak.
18. *Mycosphaerella citricola* Tilak.
On leaves of *Citrus medica* L.
Poona, 15th Aug. Leg. S. T. Tilak.
19. *Mycosphaerella heraclei* (Fr.) Petr.
On leaves of *Heracleum* sp.
Katraj, Poona, 15th Aug. 1958, Leg. T. S. Viswanathan.

20. *Mycosphaerella indica* VISWANATHAN.
On leaves of *Morus alba* L.
Poona, 15th Aug. 1958, Leg. T. S. VISWANATHAN.

21. *Mycosphaerella jasminicola* VISWANATHAN.
On leaves of *Jasminum primulinum* HEMSL.
Poona, 15th Aug 1958, Leg. T. S. VISWANATHAN.

22. *Mycosphaerella pandanii* TILAK.
On leaves of *Pandanus fascicularis* LAMB.
Poona, 10th Aug. 1958, Leg. S. T. TILAK.

23. *Mycosphaerella poonensis* VISWANATHAN.
On leaves of *Ehretia aspera* WILD.
Law College, Poona, Sept. 1958, Leg. T. S. VISWANATHAN

*24. *Ophiobolus helianthi* TILAK.
On dried stems of *Helianthus annuus* L.
Law College, Poona, July, 1959, Leg. S. T. TILAK.

*25. *Ophiobolus* sp.
On dried stems of *Duranta plumeri* FACQ.
Law College, Poona, 12th Aug. 1959, Leg. S. T. TILAK.

26. *Parodiella indica* TILAK.
On leaves of *Heylandia latibrosa* DC.
Katraj Ghat, Poona, 25th Aug. 1958, Leg. S. T. TILAK.

27. *Parodiella paraguyensis* SPEZ.
On leaves of *Desmodium triflorum* (L.) DC.
Torna Fort, Poona, 17th Aug. 1958, Leg. S. T. TILAK.

28. *Parodiella perispordiodes* (BERK. & CURT) SPEG.
On leaves of *Alysicarpus vaginalis* DC. & *Crotalaria rugosa*.
Poona, Sept.-Oct. 1958, Leg. S. T. TILAK.

29. *Parodiella spegazzinii* THEISS & SYD.
On leaves of *Crotalaria triquetra* DALZ.
Khandala, 10th Sept. 1957, Leg. S. T. TILAK.

30. *Phyllachora agharkarii* TILAK.
On leaves of *Eleocarpus oblongus* GAERTEN.
Mahabaleshwar, Jan. 1958, Leg. S. T. TILAK.

31. *Phyllachora dolichospora* SYD.
On leaves of *Tinospora cordifolia* MIERS.
Poona, July, 1958, Leg. S. T. TILAK.

32. *Phyllachora ectophytica* TILAK.
On leaves of *Mesa indica* WALL.
Mahabaleshwar, Jan. 1958, Leg. S. T. TILAK.

33. *Phyllachora glycosmis* PETCH.
On leaves of *Glycosmis pentaphylla* CORREA.
Matheran, July, 1958, Leg. S. T. TILAK.

34. *Phyllachora ortonii* TILAK.
On leaves of *Acacia suma* BUCH-HAM.
Poona, Sept. 1958, Leg. S. T. TILAK.

35. *Physalospora pandani* STEV. PIER.
On leaves of *Pandanus fascicularis* Lam.
Poona, Oct. 1958, Leg. S. T. TILAK.

36. *Pleospora arxii* TILAK.
On stems of *Cocculus villosus* DC.
Law College, Poona, June, 1959, Leg. S. T. TILAK.

37. *Pleospora mullerii* TILAK.
On leaves of *Hiptage benghalensis* KURZ.
Poona, Aug. 1958, Leg. S. T. TILAK.

38. *Salmanomyces kamatii* CHIDDARWAR.
On leaves and stems of *Acalypha ciliata* WALL.
Purandar Fort, Poona, Aug. 1958, Leg. P. P. CHIDDARWAR.

39. *Scolecodothis kamatii* TILAK.
On leaves of *Cryptolepis buchnani* R & S.
Thorna, Fort, Poona, Aug. 1958, Leg. S. T. TILAK.

*40. *Sydowia agharkarii* VISWANATHAN.
On stems of *Jasminum primulinum* HEMSL.
Law College, Poona, 9th Feb. 1959, Leg. T. S. VISWANATHAN.

41. *Thyridaria incrustans* SACC.
On bark of *Syzygium cumini* (L) SKEELS.
Poona, 9th Feb. 1959, Leg. T. S. VISWANATHAN.

42. *Tympanopsis* sp.
On stem of *Duranta plumeri* FACQ.
Poona, 12th Aug. 1959, Leg. S. T. TILAK.

FUNGI IMPERFECTI

43. *Alternaria zinniae.*
On leaves of *Ageratum conyzoides* L.
Law College, Poona, Nov. 1959, Leg. T. S. VISWANATHAN

44. *Ascochyta cycadina* SCALIA.
On leaves of *Cycus revoluta* THUMB.
Law College, Poona, Aug. 1959, Leg. T. S. VISWANATHAN

45. *Cercospora gymnosporia* VISWANATHAN.
On leaves of *Gymnosporia rothiana* M.
Law College, Poona, July, 1958, Leg. T. S. VISWANATHAN

46. *Cercospora poonensis* VISWANATHAN.
On leaves of *Ehretia aspera* WILLD.
Law College, Poona, Sept. 1958, Leg. T. S. VISWANATHAN

47. *Ciliochorella eucalypti* VISWANATHAN.
On dried leaves of *Eucalyptus globulus* LINN.
Poona, July 1958, Leg. T. S. VISWANATHAN.

48. *Cilichorella mangiferae* SYD.
On leaves of *Mangifera indica* LINN. & *Lagerstroemia flosregina* RETZ.
Poona, July, 1958, Leg. T. S. VISWANATHAN.

*49. *Cladosporium* sp.
On leaves of *Mesa indica* WALL., & *Eleocarpus oblongus* GAERTEN.
Mahabaleshwar, Jan. 1959, Leg. S. T. TILAK.

50. *Colletotrichum dematium* (PERS. ex FR.) GRAVE.
On leaves of *Calotropis gigantes* BR.
Poona, July, 1958, Leg. T. S. VISWANATHAN.

51. *Colletotrichum compactum* RAMK.
On dried pods of *Albizzia lebbek* BENTH.
Law College. Poona, July, 1959, Leg. T. S. VISWANATHAN

*52. *Colletotrichum jasminicola* TILAK.
On leaves of *Jasminum sambac* AIT.
Poona, Aug. 1959, Leg. S. T. TILAK.

*53. *Colletotrichum moricola* VISWANATHAN.
On twigs of *Morus alba* LINN.
Poona, July, 1959, Leg. T. S. VISWANATHAN.

54. *Discosia bombycina* VISWANATHAN.
On leaves of *Syzygium cumini* SKEEL.
Poona, July, 1958, Leg. T. S. VISWANATHAN.

55. *Discosia hiptages* TILAK.
On leaves of *Hiptage benghalensis* KURZ.
Law College, Poona, July, Agu. 1958, Leg. S. T. TILAK

*56. *Haplosporella* sp.
On stem of *Duranta plumeri*, FACQ.
Poona, 12th Aug. 1958, Leg. S. T. TILAK.

*57. *Oidium* sp.
On leaves of *Mesa indica* WALL.
Mahabaleshwar, Jan. 1959, Leg. S. T. TILAK.
On leaves of *Phyllanthus niruri* LINN.
Law College, Poona, Feb. 1959, Leg. T. S. VISWANATHAN.

58. *Ovularia hydrabadense* SALAM & RAO.
On leaves of *Euphorbia geniculata* ORT.
Poona, Sept. 1959, Leg. T. S. VISWANATHAN.

59. *Periconia byssoides* PERS. ex SHAW.
On pods of *Phaseolus radiatus* LINN.
Vithalwadi, Poona, Oct. 1958, Leg. S. T. TILAK.

60. *Periconia minutissima* CORDA.
On leaves of *Lobelia nicotinaefolia* HEYNE.
Mahabaleshwar, Nov. 1958, Leg. S. T. TILAK.

61. *Periconia madreeya* Subram.
On dead leaves of *Cynodon dactylon* Pers.
Vithalwadi Poona, July, 1958, Leg. S. T. Tilak.

62. *Periconia parasitica* Tilak.
On leaves of *Desmodium laxiflorum* DC.
Vithalwadi, Poona, Sept. 1957, Leg. S. T. Tilak.

63. *Pestalotia funera* Desmaz.
On leaves of *Eucalyptus globulus* Linn.
Law College, Poona, July 1959, Leg. T. S. Viswanathan.

64. *Pestalotia elasticola* P. Henn.
On leaves of *Artocarpus integrifolia* Linn.
Poona, July, 1959, Leg. S. T. Tilak.

65. *Pestalotia sapotae*, P. Henn.
On leaves of *Achras sapota* Linn.
Poona, July, 1959, Leg. S. T. Tilak.

66. *Septoria artocarpi* Cke.
On leaves of *Artocarpus integrifolia* Linn.
Poona, July, 1959, Leg. S. T. Tilak.

67. *Septoria heraclei*, Desm.
On leaves of *Heracleum* sp.
Katraj. Poona, Aug. 1959, Leg. T. S. Viswanathan.

68. *Septoria nilgierensis* Tilak.
On leaves of *Artimesia nilgirica* Pamp.
Purandhar Fort, Poona, Aug. 1958, Leg. S. T. Tilak.

References

Chiddarwar, P. P., (1958 24 Additions to Fungi of Bombay — IV. Science & Culture: 290—291.

—, (1959) Salmanomyces — A new member of Erysiphaceae. Sydowia 13: (in press.)

Cook, M. C., (1876) Some Indian Fungi. Grev. **IV** p. 114.

Mundkur, B. B. & Ahmad S., (1942) Indian & Burmese species of Pestalotia & Monochaetia. Mycl. 34: 309—317.

Petch, T., (1917) Additions to Ceylon Fungi — I. Ann. Roy. Bot. Gard. Paradenya 227.

Ramakrishnan, T. S., (1951) Additions to fungi of Madras — XI. Proc. Ind. Acad. Sci. **B-34**: 70.

Salam, A. & Rao, P. N., (1957) Fungi from Hyderabad — I. J. Ind. bot. Soc. 36: 425—426.

Stevens, F. L. & Piers, A. S., (1933) Fungi from Bombay. Ind. J. agr. Sci. 913.

Subramanian, C. V., (1955) Some species of Periconia from India, J. Ind. bot. Soc. 34: 329—361.

Sydow, H. & P. & Butler, E. J., (1911) Fungi Indiae Orientalis, Pars. III. Ann. Mycol. **IX**, 372—421.

—, (1916) Fungi Indiae Orientalis, Pars. IV, *Ibid.* **XIV**, 179.

Theissen, F. & Sydow, H., (1917) Die Gattung Parodiella. Ibid. **XV** 125—142.

Tilak, S. T., (1958) A new species of Microcyclus from India. Sydowia 12: 197—199.

—, (1958) Studies in the Indian Phyllachoroceae. Sydowia 12: 185—188.

—, (1958) A parasitic species of Periconia from India. Mycopathol. Mycol. Appl. **IX**: 194—196.

—, (1959) A new species of Septoria and its associated ascigerous stage. Mycopathol. Mycol. Appl. **X**: 216—218.

—, (1959) Second contribution towards Indian Phyllachoraceae. Sydowia 13: (in press).

—, (1959) Two undescribed species of Lophodermium from India. Sydowia (in Press).

—, (1960) Two new specis of Pleospora from India. Mycopathol. Mycol. Appl. **XII**, 141—144.

—, & Viswanathan, T. S., (1958) Two new species of Discosia from Bombay. Curr. Sci. 28: 252.

Uppal, B. N., Patel, M. K. & Kamat, M. N., (1934) Fungi of Bombay. Department of Agri. Bull. 8, 56.

Viswanathan, T. S., (1958) A new species of Erysiphe. Mycopathol. Mycol. Appl. **IX**, 150—151.

—, (1959) A new species of Cilliochorella on Eucalyptus globulus L. Curr. Sci. 28: 171.

—, (1959) A new species of Cercospora and its associated ascigerous stage Mycopathol. Mycol. Appl. **X**, 219—222.

—, (1960) A new species of Mycosphaerella from India. Mycopathol. Mycol. Appl. **XII**, 156—158.

— & Tilak, S. T., (1959) Some new records of Mycosphaerella from India. Sydowia 13: (in press).

* Descriptions of these new species will be published elsewhere.

SU DI UNA INTERESSANTE HELICOSPOREA ISOLATA DA TERRENO AD EUCALITTO: HELICOSPORINA VERONAE n. sp. IN COLTURA PURA

ANGELO RAMBELLI

(21.III.1960)

Centro di Sperimentazione Agricola e Forestale
dell' Ente Nazionale per la Cellulosa e per la Carta, Roma
Direttore prof. A. DE PHILIPPIS

Tra i numerosi microrganismi presenti nei terreni forestali, le Elicosporee non sono certamente fra quelli più diffusi e facilmente reperibili. Le difficoltà di sviluppo di questi miceti sui comuni substrati e gli stessi metodi di isolamento, fanno si che essi rappresentino una piccolissima percentuale tra tutti i numerosi generi che si rinvengono comunemente. Tanto che le maggiori e fondamentali monografie relative i funghi del terreno non ne parlano affatto.

Durante lo studio della micocenosi dei terreni coltivati ad eucalitto abbiamo trovato una Elicosporea, che ci è sembrata di un certo interesse, se non altro, sistematico. Fu allora allestita una serie di colture in capsule Petri, per studiarne i caratteri morfologici su diversi substrati: agar Czapek, malto, fagioli, carote.

I caratteri morfologici rilevati che ora passiamo a descrivere, vengono elencati secondo lo schema già adottato per altri nostri lavori di micologia.

CARATTERI DELL'APPARATO VEGETATIVO

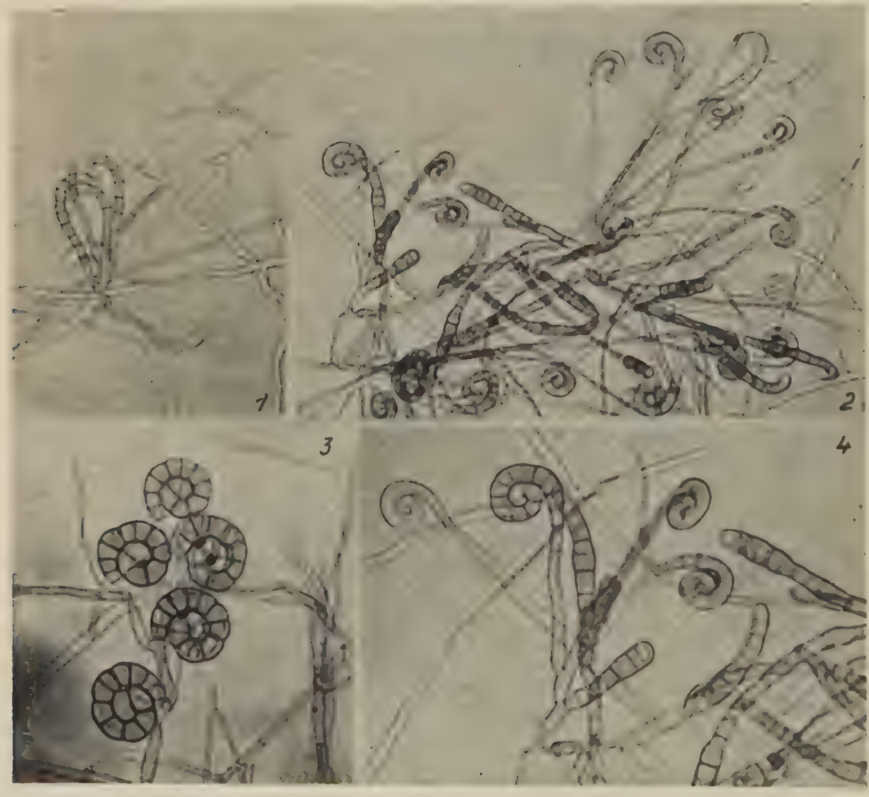
Micelio composto da ife giallo-chiare o bruno-chiare, semplici o ramificate, larghe da 2 a 3 micron, settate, a pareti e setti facilmente visibili, a contenuto protoplasmatico omogeneo, ad andamento rettilineo o leggermente sinuoso e con rigonfiamenti regolari in prossimità dei setti. Le ife si riuniscono frequentemente in fascetti di 2—5 o più elementi, oppure si avvolgono in spirali o in veri e propri anelli, larghi fino a 20— 25 micron in diametro.

Non esistono differenze morfologiche nel micelio sviluppato sui quattro substrati considerati.

Il presente lavoro rappresenta il IV Contributo alle "Schede micologiche" pubblicate dall'Autore.

CARATTERI DELL'APPARATO RIPRODUTTIVO

In agar Czapek, il conidioforo è composto da 2—4 cellule regolari, a setti e pareti facilmente visibili, leggermente rigonfiate ed allungate talvolta con inclusioni oleose. Presenta tinte meno marcate del micelio e dei conidi. E'alto fino a 20—21 micron, in prossimità della base é largo dà 4 a 5 micron. Alla base termina generalmente con leggero ingrossamento e all'apice presenta uno o più punti di inserzione dei conidi. Spesso si allunga in ramificazioni a loro volta fruttificanti. Ha un andamento eretto o delicatamente incurvato in prossimità della base. Spesso assume caratteri simili a quelli delle fruttificazioni, per cui diviene difficilmente differenziabile. Talvolta è addirittura assente e i conidi sono prodotti alternativamente sulle ife miceliari, le cui cellule assumono però caratteri cromatici e morfologici simili a quelli del conidioforo. E'prodotto sia direttamente dal substrato (dalle ife immerse), che dalle ife aeree.



Helicosporina Veronae n. sp. in coltura pura. Fig. 1, conidi nelle prime fasi di formazione (agar Czapek), X 650, fig. 2, 4. aspetto morfologico ottenuto in agar carote, X 650 e X 1000, fig 3. aspetto morfologico normale ottenuto in agar fagioli, X. 1000.

Conidi spiralati, formati da una ifa che si avvolge anche ripetutamente su se stessa. Sono disposti su un piano e solo raramente le cellule interne (le più giovani) coprono parzialmente le più esterne. Sono bruno chiari, larghi da 15 a 18 micron in diametro. Sono composti da 14—18 cellule rigonfiate, che si allargano fino a 5 micron procedendo dalla base fino all'apice della spirale. Pareti e setti facilmente visibili, a contenuto protoplasmatico omogeneo, più raramente granuloso, o con inclusioni oleose. Sono prodotti in numero limitato (2 o 3) all'apice dei conidiofori, da sorta di sterigmi fruttificanti, che si formano successivamente e lateralmente al primo conidio prodotto. Assumono forme diverse: possono ripiegarsi fino dalle prime fasi di sviluppo e formare spirali, oppure assumere l'andamento regolare dopo essersi innalzati verticalmente per qualche micron sull'apparato fruttificante.

I caratteri descritti sono identici a quelli rivelati in agar-fagioli.

In agar-carote: fruttificazioni abbondanti. Conidi e conidiofori giallo-chiari. Questi ultimi notevolmente e spesso irregolarmente allungati. Conidi talvolta non ripiegati a spirale, con cellule regolari disposte in successione verticale, tanto da assumere l'aspetto di quelli tipici del genere *Sporidesmium*. Oppure le cellule apicali si ripiegano per formare una sorta di uncino, dopo un lungo decorso decisamente rettilineo; in generale i conidi non assumono un aspetto così serrato, come quello riscontrato in agar Czapek e fagioli.

In agar-malto, fruttificazioni regolari. Conidi bruno-chiari che raggiungono fino a 20 micron di diametro. I conidiofori generalmente presentano le normali forme e dimensioni rivelate in agar Czapeke fagioli, talvolta però sono notevolmente e sproporzionatamente allungati.

CARATTERI DELLE COLONIE NEI SUBSTRATI ARTIFICIALI

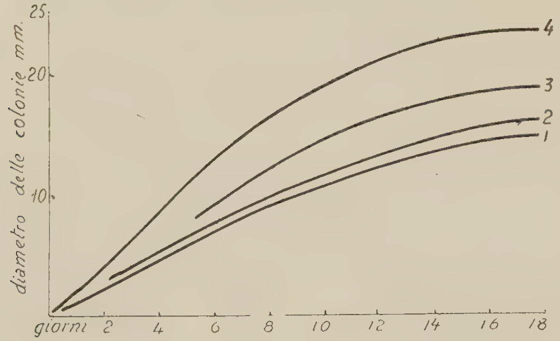
In agar Czapek sviluppa stentatamente, formando, dopo 15 giorni di coltura, colonie bruno-scure inferiormente, con tinte meno marcate superiormente. Sono feltrose, sollevate fino a mm 3 sul substrato, con margini più chiari e a sviluppo notevolmente dendritico.

In agar-fagioli, carota e malto, sviluppo più abbondante e regolare. Colonie con le stesse caratteristiche descritte precedentemente, senza sviluppo dendritico marginale.

L'accrescimento nel tempo delle colonie in vari agar e a 26°C è rappresentato dalla annessa figura.

IDENTIFICAZIONE

Il microrganismo descritto presenta caratteri tali da rendere la sua determinazione di una certa difficoltà. La presenza di conidi disposti in spirale, localizzati all'apice di conidiofori semplici o ramificati, ci ha portati a considerarlo inizialmente un *Helicoma*. Ma in *Helicoma* i conidi non sono così serrati e compatti ed i conidio-



Sviluppo delle colonie in piastra alla temperatura di 26° C. su agar-Czapek(1), agar-malto (2), agar-carote(3), agar-fagiuoli(4)

fori sono decisamente pigmentati di bruno scuro, contrariamente a quanto si verifica nel nostro micete. Così pure abbiamo scartato *Helicosporium* per le fruttificazioni prodotte lateralmente al conidioforo preformato. Anche il genere *Xenosporella*, nel quale avevamo provvisoriamente incluso il nostro micete, non può essere considerato rispondente per la presenza nei conidi di settazione longitudinale e trasversale. Rispondente alle caratteristiche descritte ci è sembrato invece il genere *Helicosporina*. Questo Genere fu istituito da G. ARNAUD nel 1953 per includere quei micromiceti che presentano conidioforo ben sviluppato con produzione apicale di conidi spiralati.

In effetti la diagnosi data da ARNAUD per il genere, come pure per l'unica specie fino ad oggi nota, l'*Helicosporina globulifera* ARN., ci è apparsa quanto mai scarsa o per lo meno insufficiente per poter determinare ed includere anche microrganismi studiati sui comuni substrati da coltura. D'altra parte il nostro microrganismo non è privo di importanza, perchè viene a confermare, come seconda specie, il genere *Helicosporina* di ARNAUD. Infatti siamo propensi a considerarlo una nuova specie, avendo *l'H. globulifera* ARN. caratteri dimensionali sostanzialmente differenti.

In ultima analisi riteniamo utile, oltre alla diagnosi specifica ripetere ed arricchire di caratteri anche la diagnosi generica secondo quanto segue:

Helicosporina ARNAUD. Micelia ab hyphis dilute brunneis vel dilute flavis composita, simplicibus vel in ramo diffusis, vel in fascibus conglobatis. Parietibus et septis plane manifestis.

Conidiophora cylindracea, dilute brunnea, vel dilute flava, simplicia, vel ramosa, septata.

Conidia dilute brunnea, spiralata pressata, in uno plano disposita, in apice evolvente, et in apice conidiophorum in successione acrogena producta.

Helicosporina Veronae n. sp. — Hyphis mycelicis dilute brunneis, vel pallentibus, 2—3 micron latis, parietibus et septis clare manifestis, protoplasmate homogeneo, simplicis, vel in ramo diffusis, vel in fascibus conglobatis.

Conidiophoris pallentibus erectis, simplicis, vel ramosis, usque ad 21 micron longis et usque ad 5 micron latis prope basi. Ab 2—5 cellulis plane delineatis compositis. Parietibus et septis clare manifestis, protoplasmate homogeneo, vel oleosis inclusionibus. Apice mono vel pluri fertile.

Conidiis dilute brunneis 15—20 micron latis, 14—17 cellulis compositis, parietibus et septis plane manifestis et oleosis inclusionibus. Spiralatis, pressatis, in uno plano dispositis.

Habitat — In humo sub plantis Eucalypti. Italia Centrale. Latio. Prope Romam.

Per le caratteristiche descritte *l'Helicosporina Veronae* n. sp. deve essere inclusa nella II sezione della classificazione di Hughes.

RIASSUNTO

L'A. descrive e determina sistematicamente una nuova specie di *Helicosporina* (*Helicosporina Veronae* n. sp.), rinvenuta nel Lazio in terreni coltivati ad eucalitto.

I caratteri tassonomici riguardano gli organi vegetativi e riproduttivi ottenuti in substrati artificiali.

SUMMARY

The Autor describes and systematically determines a new species of helicosporous fungi (*Helicosporina Veronae*, n. sp.), found in Lazio in Eucalyptus soils.

The morphological characters refer to the reproductive and vegetative organs in artificial mediums.

BIBLIOGRAFIA

Arnaud, G., 1953 .*Mycologie concrete Genera II* Bull. Soc. Mycol. France. **69**: 265—306.

Davidson, A. M. & Gregory, 1938. *The spiral hyphae of Trichophyton* Trans. Brit. Mycol. Soc. **21**: 98—113.

Clen Bott, I. Janet, 1955. *On Helicodendron tubulosum and some similar species* Trans. Brit. Mycol. Soc. **38**: 17—30.

Gilman, J. C., 1957. *A manual of soil fungi* Ames Iowa,

Hughes, S. J., 1953 *Conidiophores, conidia and classification* Canad. J. Bot. **31** 577—659.

Desidero ringraziare vivamente Mons. Ottorino Alberti della Pontificia Università Lateranense per avemi riveduto la diagnosi latina.

HUGHES, S. J., 1958 *Revisiones hyphomicetum aliquot cum appendice de nominibus rejiciendis* Canad. J. Bot. **36,** 727—836.

LINDER, D. H., 1933. *North American Hyphomycetes I Two new Helicosporeae and the new genera Haplochalara and Paspalomyces* Mycologia **25**: 342—348.

MOORE, T.R., 1955. *Index to the Helicosporae* Mycologia **47**: 90—103.

MOORE, T. R., 1957. *Index to the Helicosporae Addenda,* Mycologia **49**: 580—587.

NIETHAMMER, A., 1937. *Die Mikroskopischen Bodenpilze* ad. D. W. Junk, L'Aja.

RAMBELLI, A., 1959 *Schede micologiche III Contr. Micromiceti della Valle del Tevere.* Mycopathol. Mycol. appl. **11**: 135—142.

VAN BEVERWIJK, L. AGATHE, 1953 *Helicosporous hyphomycetes* Trans. Brit. Mycol. Soc. **36**: 111—124.

GROWTH OF DIMORPHIC HUMAN PATHOGENIC FUNGI ON MEDIA CONTAINING CYCLOHEXIMIDE AND CHLORAMPHENICOL

BY

E. S. Mc Donough, Ph. D., Lucille K. Georg, Ph. D., Libero Ajello, Ph. D. & Sherry Brinkman[1])

(21.III.1960)

INTRODUCTION

It was recently reported (1) that several strains of *Blastomyces dermatitidis* were more sensitive to cycloheximide and chloramphenicol at 37° C than at 25° C. It was also observed that a similar temperature sensitivity differential existed for certain other pathogenic fungi.

Cycloheximide is widely used for the selective isolation of human pathogenic fungi from materials contaminated with saprophytic fungi (2—9) and chloramphenicol is employed to inhibit bacterial contaminants (8,10). For these reasons further studies were undertaken to determine the limitations in the use of these antibiotics in isolation media and in the preservation and shipment of clinical materials.

MATERIALS AND METHODS

Twenty-four strains of *B. dermatitidis*, five strains of *Histoplasma capsulatum*, five strains of *Paracoccidioides brasiliensis* and five strains of *Sporotrichum schenckii* were tested for sensitivity to cycloheximide and chloramphenicol at 37° C and 25° C. These fungi were grown on two sets of brain heart infusion agar slants, one containing serial dilutions of cycloheximide and the other chloramphenicol.

Unless otherwise stated, the minimum number of yeast-phase cells, which consistently gave good growth on plain Brain Heart Infusion Agar at 37° C, was used as the inoculum. Each ml of the stock suspension used for inoculation contained approximately 600,000 cells of *B. dermatitidis*, 3,600,000 cells of *H. capsulatum*, 3,000,000 cells of *S. schenckii* and 2,400,000 cells of *P. brasiliensis*.

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The following amounts were used to inoculate the culture tubes: *B. dermatitidis* 0.01 ml, *S. schenckii* 0.01 ml, *H. capsulatum* 0.2 ml and *P. brasiliensis* 0.2 ml. The cultures were read at weekly intervals over a 3 week period of incubation.

RESULTS

Cycloheximide. Results obtained with the 24 strains of *B. dermatitidis*, studied in regard to cycloheximide sensitivity, confirm the general observations previously presented as to yeast phase mycelial phase sensitivity (1). The yeast-phase was consistently more sensitive to cycloheximide than the mycelial phase. This was true regardless of whether mycelial cells (conidia, chlamydospores and mycelial fragments) or yeast cells were used as inocula. The growth produced, however, was consistently lower at 37° C when mycelial cells were used as the inoculum (Fig. 1).

Considerable individual variation in the sensitivity of the various strains to cycloheximide was discovered. Of the 24 strains studied, 3 failed to grow at 37° on media containing as little as 0.025 mg/ml of cycloheximide. The growth produced by a "sensitive" and a "resistant" strain is indicated in Fig. 2.

Sensitivity to cycloheximide was also influenced by inoculum size (Table I). When approximately 600,000 yeast cells of the "J" strain were used to inoculate media containing 0.8 mg/ml cycloheximide growth after 3 weeks at 37° C was similar to that of the control. Only a trace of growth occurred, however, when the same medium was inoculated with 6,000 cells per tube. Similar results were obtained with several other strains. The basis for the greater resistance of the concentrated inocula to cycloheximide is not known.

In order to determine if resistance could be developed, the following experiments were performed: Strain "CO" of *B. dermatitidis* produced only sparse growth on tubes of BHI agar containing 0.05 mg cycloheximide per ml of medium when 6,000 cells were used in the inoculum. All the growth obtained from one such tube was inoculated onto a second tube of agar containing 0.05 mg/ml cycloheximide. At the end of 2 weeks all the growth on this tube was transferred to B.H.I. agar containing 0.1 mg/ml cycloheximide. Subsequently the growth was transferred every two weeks to a more concentrated cycloheximide medium so that at the end of the experiment the growth had been left for two weeks each on concentrations of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, and 2.0 mg per ml of cycloheximide. At the end of this series of transfers abundant growth was produced on the medium contraining 2.0 mg/ml of cycloheximide when inoculated with 6,000 cells. It has not been established whether or not the increase in resistance was caused by selection of resistant cells or by the development of "adaptive enzymes".

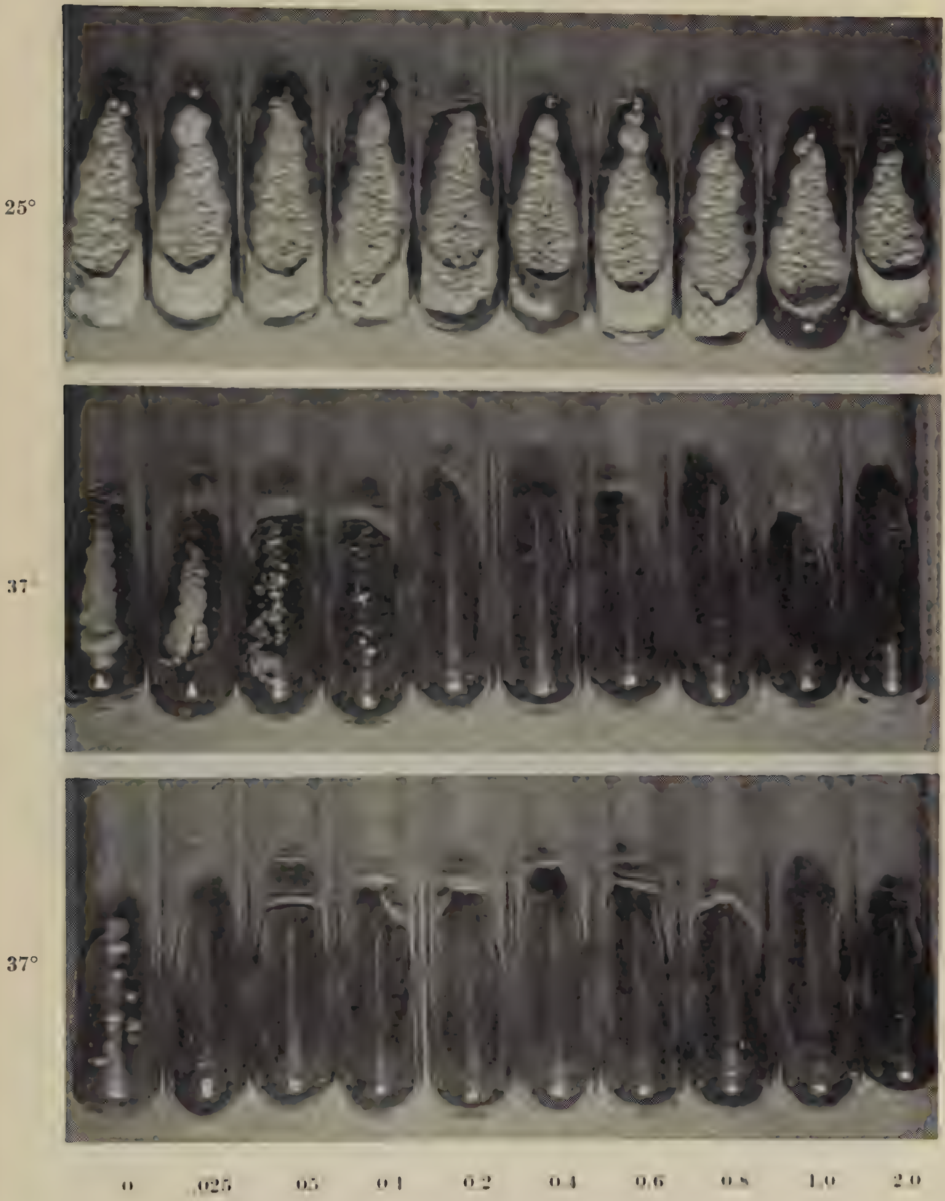

Fig. 1. Effects of temperature on growth of *Blastomyces dermatitidis* (strain H) on B.H.I. *Cycloheximide* agar (21 days). The numbers below the figures indicate mg/ml. The bottom row of tubes was inoculated with mycelial cells, the upper rows with yeast cells, the approximate number of mycelial cells or yeast cells per inoculum being the same.

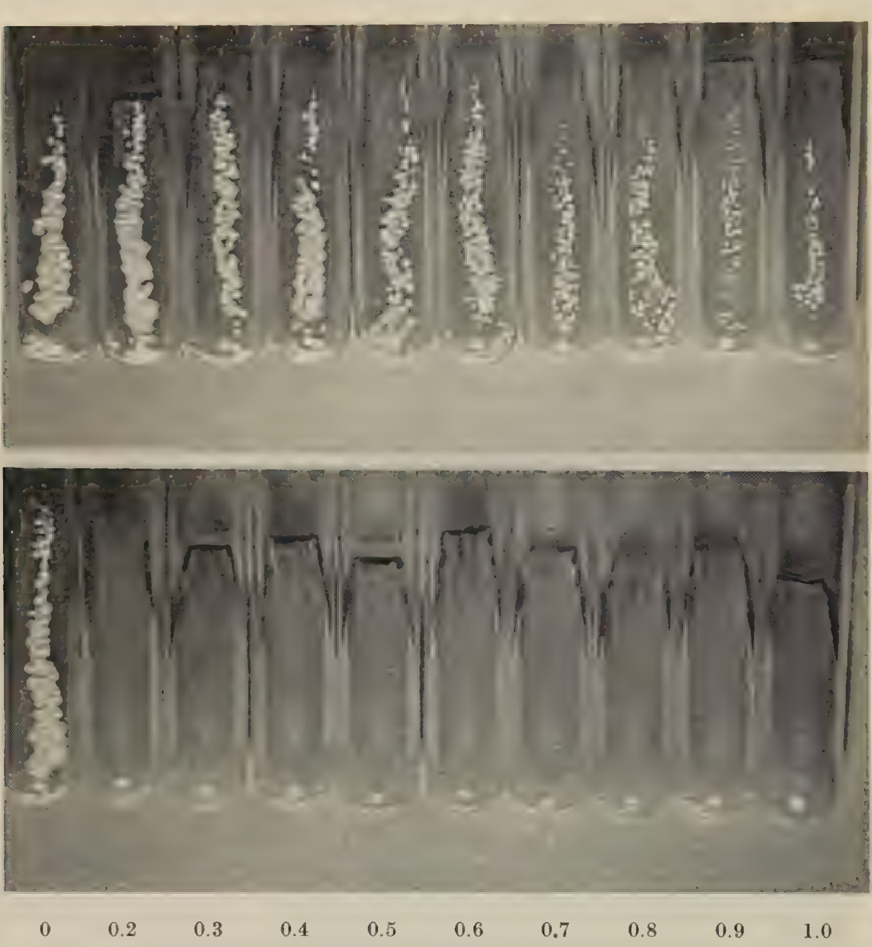

Fig. 2. Sensitivity of *Blastomyces dermatitidis* (strain A above, strain H below) to cycloheximide — eleven days at 37° C. The numbers below the figure indicate mg/ml.

The results obtained with the other species (*H. capsulatum, P. brasiliensis, S. schenckii*) were essentially similar to those obtained with *B. dermatitidis.*

In Table II the growth of the most sensitive and the most resistant strains of *B. dermatitidis, H. capsulatum, P. brasiliensis*, and *S. schenckii* on B.H.I. cycloheximide agar are recorded. It would appear that *B. dermatitidis* is much more sensitive than any one of the other three fungi studied, especially in the yeast phase at 37° C. This may not be true, however, since larger numbers of cells were required for inoculum in the case of these later organisms. Also 24 strains of *B. dermatitidis* were studied and only 5 of each of the other fungi.

TABLE I.

Influence of the number of yeast cells in the inoculum on survival and growth of the "J" strain of Blastomyces dermatitidis (Cycloheximide B. H. I. Agar) (37° C)

Antibiotic Conc. mg/ml	Number of Cells and Weeks of Growth								
	600.000			60.000			6.000		
	1	2	3	1	2	3	1	2	3
2.0	+	1	3	—	—	+	—	—	—
1.0	+	3	3	—	—	+	—	—	—
0.8	+	3	4	—	+	+	—	—	+
0.6	+	3	4	+	+	1	—	+	+
0.4	+	3	4	+	+	1	—	+	+
0.2	+	3	4	+	+	1	—	+	+
0.1	1	3	4	+	+	1	+	+	+
0.05	1	3	4	+	+	1	+	+	1
0.025	1	3	4	+	+	2	+	+	2
Control	4	4	4	4	4	4	4	4	4

Growth in control tubes containing no antibiotic was considered as 4 at the time of each reading. + stands for trace.

TABLE II.

Growth of Sensitive (S) and Resistant (R) Strains of Paracoccidioides brasiliensis, Histoplasma capsulatum, Sporotrichum schenckii and Blastomyces dermatitidis after three Weeks on B. H. I. Agar

Antibiotic Conc. mg/ml	*P. brasiliensis*				*H. capsulatum*				*S. schenckii*				*B. dermatitidis*			
	S-Strain		R-Strain		S-Strain		R-Strain		S-Strain		R-Strain		S-Strain		R-Strain	
	37°	25°	37°	25°	37°	25°	37°	25°	37°	25°	37°	25°	37°	25°	37°	25°
2.0	+	2	1	2	—	1	—	1	—	4	2	4	—	2	3	3
1.0	+	3	2	3	—	2	+	2	—	4	3	4	—	3	4	4
0.8	1	3	3	4	1	3	1	3	1	4	4	4	—	3	4	4
0.6	1	4	3	4	1	4	2	3	2	4	4	4	—	3	4	4
0.4	2	4	3	4	2	4	2	3	3	4	4	4	—	4	4	4
0.2	3	4	3	4	2	4	3	4	4	4	4	4	—	4	4	4
0.1	4	4	4	4	3	4	4	4	4	4	4	4	—	4	4	4
0.05	4	4	4	4	3	4	4	4	4	4	4	4	—	4	4	4
0.025	4	4	4	4	4	4	4	4	4	4	4	4	—	4	4	4
Control	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

Chloramphenicol. The same 24 strains of *B. dermatitidis*, 5 strains of *H. capsulatum*, 5 of *P. brasiliensis* and 5 of *S. schenckii* used in the cycloheximide studies were assayed in regard to sensitivity to chloramphenicol.

In Table III is recorded for each species the growth of the most sensitive and most resistant strain. The most sensitive strain of both *P. brasiliensis* and of *S. schenckii* was shown to be more sensitive than that of *B. dermatitidis* at 37° C. The difference on a cell per cell basis however might be greater since fewer cells were used in the inoculation of *B. dermatitidis*. The comparatively small number of strains assayed (5) may have been the reason a more sensitive strain of *H. capsulatum* was not found. Also, a comparatively large number of cells (720,000) was used for the inoculation of this organism.

TABLE III.

Growth of Sensitive (S) and Resistant (R) Strains of Paracoccidioides brasiliensis, Histoplasma capsulatum, Sporotrichum schenckii and Blastomyces dermatitidis after three Weeks on B. H. I. Chloramphenicol Agar

Antibiotic Conc. mg/ml	*P. brasiliensis*				*H. capsulatum*				*S. schenckii*				*B. dermatitidis*			
	S-Strain		R-Strain		S-Strain		R-Strain		S-Strain		R-Strain		S-Strain		R-Strain	
	37°	25°	37°	25°	37°	25°	37°	25°	37°	25°	37°	25°	37°	25°	37°	25°
0.6	—	—	+	3	—	2	+	2	—	4	1	4	—	3	1	2
0.4	—	2	+	4	—	3	1	3	—	4	3	4	—	3	3	3
0.2	—	4	2	4	1	4	2	4	—	4	4	4	—	4	4	4
0.1	—	4	3	4	1	4	3	4	—	4	4	4	—	4	4	4
0.08	—	4	4	4	2	4	4	4	+	4	4	4	+	4	4	4
0.06	—	4	4	4	2	4	4	4	+	4	4	4	1	4	4	4
0.04	1	4	4	4	3	4	4	4	+	4	4	4	1	4	4	4
0.02	1	4	4	4	4	4	4	4	+	4	4	4	2	4	4	4
0.01	2	4	4	4	4	4	4	4	1	4	4	4	3	4	4	4
0.005	3	4	4	4	4	4	4	4	2	4	4	4	4	4	4	4
0.0025	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4
Control	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

DISCUSSION

In general, media used for the isolation of human pathogenic fungi in which these antibiotics are incorporated usually contain 0.05 mg/ml chloramphenicol and 0.4 or 0.5 mg/ml cycloheximide. The results of these experiments indicate that some strains of *B. dermatitidis* might not be isolated at 37° C from sputum and other clinical specimens containing these concentrations of cycloheximide. It would also seem that some strains of *H. capsulatum*, *P. brasiliensis* and *S. schenckii* would not be recovered unless the inoculum contained large numbers of cells. On the other hand, no evidence was found that failure of isolation of any of the four fungi would occur at 25° C because of the cycloheximide concentration in media containing as high as 0.5 mg/ml of the antibiotic. Actually, one of the sensitive strains of *B. dermatitidis* was isolated from a patient on a standard cycloheximide, chloramphenicol medium incubated at 25° C.

The results of these experiments indicate that media containing chloramphenicol at 0.05 mg/ml will not permit isolation of some strains of each of the four fungi at 37° C. where the inoculum contains the minimum number of cells capable of producing growth on plain B.H.I. agar. Such failure would not occur, however, at 25° C.

It is the general practice in many clinical laboratories to preserve sputum for the subsequent isolation of certain pathogenic fungi by using chloramphenicol at concentrations ranging from 0.05 mg/ml to 0.5 mg/ml. With the possible exception of two strains of *P. brasiliensis*, the results of the present study indicate that such concentrations would not prevent the survival at room temperature of the strains of the fungi studied in sputum to which as much as 0.5 mg/ml of chloramphenicol had been added. In general practice, however, it would seem advisable to use a concentration of not more than 0.2 mg/ml of chloramphenicol in preserving sputum.

SUMMARY

Studies of 24 strains of *Blastomyces dermatitidis* confirmed previously published results that the yeast-phase of this fungus is more sensitive than the mycelial-phase to cycloheximide and chloramphenicol.

Studies of 5 strains each of *Histoplasma capsulatum*, *Paracoccidioides brasiliensis* and *Sporotrichum schenckii* show that these species also have a similar yeast-phase mycelial -phase sensitivity differential in regard to these antibiotics.

A cycloheximide resistant strain of *B. dermatitidis* was developed from a sensitive strain.

The experimental results support the general practice of using 0.5 mg/ml cycloheximide and 0.05 mg/ml chloramphenicol in media for the isolation of the four fungi at 25° C. The results indicate, however, that some strains would not be recovered at 37° C with similar concentrations of these antibiotics.

It is recommended that a concentration of not more than 0.2 mg/ml chloramphenicol should be used to preserve sputum which is subsequently to be cultured for *B. dermatitidis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis* or *S. schenckii*.

ACKNOWLEDGEMENT

E. S. McDonough and Sherry Brinkman were supported in the investigation by Research Awards E2211 and E2211 (C1) from the National Institutes of Allergy and Infectious Diseases, Public Health Service, to Marquette University.

REFERENCES

1. McDonough, E. S., Ajello, L., Georg, L. K. & Brinkman, S. (1960) In vitro effects of antibiotics on the yeast phase of Blastomyces dermatitidis and other fungi, J. Lab. & Clin. Med **50**, 116—119.

2. FUENTES, C. A., TRESPALACIOS, F., BAQUERO, G. F. & ABOULAFIA, R. (1952) Effects of acti-dione on mold contaminants and on human pathogens, Mycologia **44,** 170—175.
3. GEORG, L. K. (1953) Use of a cycloheximide medium for isolation of dermatophytes from clinical materials, Arch. Derm. Syph. **67,** 355—361.
4. AJELLO, L. & GETZ, M. E. (1954) Recovery of dermatophytes from shoes and shower stalls, J. Invest. Derm. **22,** 17—21.
5. SHARVILL, D. & TALBOT, J. M. (1954) Cycloheximide in the isolation of dermatophytes, Brit. J. Derm. **66,** 214—217.
6. FEGELER, F. (1956) Untersuchungen zur Epidemiologie der Epidermophytien mit besonderer Berücksichtigung des *Trichophyton rubrum* (Castellani), Arch. klin. exp. Derm. **203,** 570—576.
7. ROSENTHAL, S. A., BAER, R. L., LITT, L.Z., ROGACHEFSKY, H. & FURNARI, D. (1956) Studies on the dissemination of fungi from the feet of subjects with and without diseases of the feet, J. Invest. Dermat. **26,** 41—47.
8. ROSENTHAL, S. A. & FURNARI, D. (1957) The use of cycloheximide-chloramphenicol medium in routine culture for fungi, J. Invest. Derm. **28,** 367—371.
9. ADAM, W. & STEITZ, K. (1958) Zur Wirkung von Cycloheximid auf Pilzkulturen, Z. Haut-u. Geschlechtskr. **25,** 153—161.
10. COUDERT, J. & MURAT, M. (1954) Intéret du milieu de Sabouraud à la chloramycétine pour l'isolement des dermatophytes et des champignons levuriformes. Ann. Biol Clin. **12**(3—4), 185—186.

MYCOLOGICAL STUDY OF 519 CASES OF RINGWORM INFECTIONS IN PORTUGAL

Significance of multiple localizations. Tinea as a single infection.

by

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(22.III.1960)

Dermatophytes have only exceptionally been demonstrated in living tissue. Keratinophily is their main characteristic and limits normally tinea infections to localizations where keratinized material is readily available. These localizations are so nearly related by clinical, biological and epidemiological features that considering them as particular aspects of the same infection seems to be justified. That is the reason for which the study of different forms of tinea must be made as a whole when we want to get a general view of such infection. Then, the designation of tinea may be given to any superficial infection caused by dermatophytes on the skin and its appendages, independently of its clinical form and localization.

As has been pointed out in a recent review of the mycological literature (1), Portuguese work on tinea has largely been concerned with the dermatophytes responsible for tinea capitis. The etiology of the other forms has been studied by OLIVEIRA (2 to 9), who identified the agents of 50 cases of tinea barbae (*Trichophyton roseum* in 90 %, *Trichophyton cerebriforme* in 6 % and *Trichophyton violaceum* in 4 %) and of 9 cases of tinea corporis (*T. violaceum* in 7, *T. roseum* in 1 and *Trichophyton schoenleinii* in 1). He also isolated for the first time in this country a strain of *Microsporum gypseum* from human ringworm. However, a systematic study of the etiology of ringworm in its various localizations had not yet been done.

The mycological study of all cases of tinea diagnosed in the departments of dermatology of Curry Cabral Hospital and Universitary Clinic in Lisbon was started in January 1959. An account of the results so far obtained is given here.

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[1]) Supported by a grant of the „Instituto de Alta Cultura", Ministry of Education.

MATERIAL AND METHODS

572 patients with tinea of the scalp, glabrous skin, nails, beard, groins, soles and toes were observed.

Infected material was removed from the lesions for direct examination in 40 % potassium hydroxide and for culture. At first two selective media for dermatophytes were used: Sabouraud's dextrose agar with penicillin, streptomycin and cycloheximide (10) and Littmann-oxgall-agar "Difco". The cultures were incubated at 24° C. Contamination by bacteria and saprophytic molds seemed more frequent with Littmann-oxgall-agar. Since this medium changes the cultural characteristics of the dermatophytes, Sabouraud's dextrose agar with antibiotics was prefered. The identification of the isolates was based on its macro- and microscopical characteristics. The nomenclature referred to by CONANT (11) was adopted. Whenever difficulties arised in the identification of isolates, nutritional tests were run according to GEORG's methods (12). Subcultures were done on the following media: a) casein agar; b) casein agar with inositol; c) casein agar with thiamin; d) casein agar with inositol and thiamin; e) ammonium nitrate agar. They were incubated at 24° C. and examined after one and two weeks. The amount of growth was recorded following GEORG's criterion: (—) no growth; (±) some growth into the subsurface of the medium and (1+ to 4+) growth on the surface of the medium evaluated by comparison.

RESULTS

Mycological study revealed the presence of fungi in the lesions of 572 patients. Cultures were obtained from 519 cases (90.7 %). Table I summarizes 647 mycological studies of these cases: 81.3 %

TABLE I.

Positive mycological examinations

Localizations	Positive direct examination Positive culture	Negative direct examination Positive culture	Positive direct examination Negative culture	Total
Scalp	283	—	12	295
Nails	60 ¹)	10	14	84
Glabrous skin	68	14	10	92
Beard	18	5	3	26
Groins	39 ²)	3	10	52
Soles	41	11	11	63
Toe interspaces	17 ²)	13	5	35
Total	526 (81.3 %)	56 (8.7 %)	65 (10.0 %)	647

¹) yeastlike cells in 2 cases respectively produced by *Candida albicans* and *Scopulariopsis* sp.

²) yeastlike cells in 1 case by *Candida albicans*.

were positive on direct examination and culture; 8.7 % were negative on direct examination and positive on culture; 10.0 % were positive on direct examination and negative on culture.

The direct examination of scales from soles and toe interspaces revealed rather frequently the presence of "mosaic fungi". These have not been detected in another material. As "mosaic fungi" were seen both in cases with and without actual demonstration of fungi (Table II), no diagnostic value was attached to them.

TABLE II.

"Mosaic fungi" (m.f.) on direct examination related to the positive cultures of dermatophytes obtained from the same material.

Localization	Positive direct examination Positive culture		Positive direct examination Negative culture		Negative direct examination Positive culture		Negative direct examination Negative culture	
	total	m.f.	total	m.f.	total	m.f.	total	m.f.
Soles	41	4	11	2	11	4	45	7
Toe interspaces . . .	17	1	5	3	13	5	41	22
Total.	58	5	16	5	24	9	86	29

TABLE III.

Rate growth of the different species:

Species	Number of cases	Incubation time (days)	
		limits	average
T. violaceum	9	4 to 19	12
T. tonsurans	12	2 to 12	9
T. megninii	10	5 to 17	9
T. rubrum	6	3 to 7	5
T. schoenleinii	2	4	4
T. mentagrophytes . . .	32	2 to 12	6
M. canis	35	2 to 12	5
E. floccosum	25	3 to 14	8

TABLE IV.

Nutritional tests with dermatophytes:

Species	Number of cases	Casein	Casein inositol	Casein thiamin	Casein inositol thiamin	NO3 NH4
T. violaceum	15	— to ±	— to ±	4 +	4 +	— to ±
T. tonsurans.	22	— to ±	— to ±	4 +	4 +	— to ±
T. megninii	36	4 +	4 +	4 +	4 +	— to ±
T. rubrum	27	4 +	4 +	4 +	4 +	2 +
T. mentagrophytes	15	4 +	4 +	4 +	4 +	2 +
T. schoenleinii	2	4 +	4 +	4 +	4 +	— to ±
T. verrucosum	1	—	±	±	4 +	—
M. canis	1	4 +	4 +	4 +	4 +	4 +
E. floccosum.	7	4 +	4 +	4 +	4+	±

TABLE V.

Fungi cultured from 519 cases of tinea infections:

Localization	Total	DERMATOPHYTES																		OTHER FUNGI			
		T. violaceum		*T. tonsurans*		*T. megninii*		*T. rubrum*		*T. mentagrophytes*		*T. schoenleinii*		*T. verrucosum*		*M. canis*		*E. floccosum*		*Candida albicans*		*Scopulariopsis*	
		cases	%	cases	%	cases	%	cases	%	cases	%	cases	%	cases	%	cases	%	cases	%	cases	%	cases	%
Scalp	275	110	40.0	46	16.7	2	0.7	—	—	1	0.4	17	6.2	—	—	99	36.0	—	—	—	—	—	—
Nails	41	13	31.7	3	7.3	13	31.7	5	12.2	4	9.8	1	2.4	—	—	—	—	1	2.4	—	—	1	2.4
Glabrous skin	46	8	17.4	14	30.4	4	8.7	4	8.7	4	8.7	—	—	—	—	11	23.9	1	2.2	—	—	—	—
Beard	20	4	20.0	3	15.0	11	55.0	—	—	1	5.0	—	—	1	5.0	—	—	—	—	—	—	—	—
Groins	25	—	—	—	—	—	—	9	36.0	1	4.0	—	—	—	—	—	—	14	56.0	1	4.0	—	—
Soles	37	1	2.7	—	—	—	—	1	2.7	29	78.3	—	—	—	—	1	2.7	5	13.5	—	—	—	—
Toe interspaces	9	—	—	—	—	—	—	1	11.1	8	88.8	—	—	—	—	—	—	—	—	—	—	—	—
Multiple lesions	66[1])	9	13.6	7	10.7	13	19.7	11	16.6	13	19.7	—	—	—	—	—	—	12	18.2	1	1.5	—	—
Total	519	145	27.9	73	14.0	43	8.3	31	6.0	61	11.7	18	3.5	1	0.2	111	21.4	33	6.3	2	0.4	1	0.2

[1]) In 11 cases, only a positive culture from one site was obtained, although direct examination was positive in all the localizations.

The rate of growth was registered for several species (Table III). *M. canis*, *T. mentagrophytes*, *T. rubrum* and *T. schoenleinii* tended to grow faster than *T. violaceum*, *T. tonsurans*, *T. megninii* and *E. floccosum*. However a great variability was observed within different isolates of the same species.

The nutritional study of 126 dermatophyte isolates (Table IV) confirmed GEORG's results (12). The method was particularly useful in the identification of *T. tonsurans* which is stimulated by thiamin, *T. verrucosum* which is stimulated by inositol and thiamin and *T. megninii* which for its requirements of 1-histidine does not develop in ammonium nitrate agar. *T. megninii* was isolated with much more frequency than generally expected and it is considered in another publication (13).

In table V the fungi obtained from 519 cases of tinea infections are listed according to their localization. They are by order of frequency:

T. violaceum (27.9 %); *M. canis* (21.4 %); *T. tonsurans* (14.0 %); *T. mentagrophytes* (11.7 %); *T. megninii* (8.3 %); *E. floccosum* (6.3 %); *T. rubrum* (6 %); *T. schoenleinii* (3.5 %); *T. verrucosum* (0.2 %). *Candida albicans* (0.4 %) and *Scopulariopsis* sp. (0.2 %) are additionally recorded.

TABLE VI.

Etiological agents of 66 *forms with multiple localization*:

(N: nails; Sk: skin; Sc: scalp; B: beard; G: groins; F: soles; I: toe interspaces)

Localization	Total of cases	AGENTS						
		T. violaceum	*T. tonsurans*	*T. megninii*	*T. rubrum*	*T. mentagrophytes*	*E. floccosum*	*C. albicans*
N & Sk . . .	14	3 2)	2 1)	5 1)	4	—	—	—
N & Sc . . .	3	3	—	—	—	—	—	—
N & F. . . .	2	—	—	—	—	2	—	—
N & I	5	—	—	1	2	1	—	1
Sk & Sc . . .	1	—	1	—	—	—	—	—
Sk & B . . .	2	—	1	1	—	—	—	—
Sk & G . . .	10	—	—	3 1)	3	—	4	—
Sk & I. . . .	2	—	—	1	1	—	—	—
G & F . . .	4	—	—	—	1	—	3 1)	—
G & I	5	—	—	—	—	1 1)	4	—
F & I	9	—	—	—	—	9 3)	—	—
N, Sk & Sc .	4	3	1	—	—	—	—	—
N, Sk & B. .	1	—	1	—	—	—	—	—
N, Sk & F. .	1	—	1 1)	—	—	—	—	—
N, Sk & I . .	1	—	—	1	—	—	—	—
N, F & I . .	1	—	—	1	—	—	—	—
Sk, G & I . .	1	—	—	—	—	—	1	—
Total.	66	9	7	13	11	13	12	1

1) in 1 case positive direct examination and negative culture in one site;

2) in 2 cases positive direct examination and negative culture in one site;

3) in 3 cases positive direct examination and negative culture in one site;

A group of forms with multiple localization, is also considered here. Table VI summarizes the observations on those multiple localizations and their causative agents. Each localization including the multiple ones was registered in the following percentage:

	number of cases	%
Scalp	283	47.6
Glabrous skin	83	14.0
Nails	73	12.3
Soles	54	9.1
Groins	45	7.6
Toe interspaces	33	5.5
Beard	23	3.9

1. Tinea capitis. From 283[1]) cases (including 8 with lesions in other sites) the following species were identified:

	number of cases	%
T. violaceum	116	41.0
M. canis	99	35.0
T. tonsurans	48	17.0
T. schoenleinii	17	6.0
T. megninii	2	0.7
T. mentagrophytes	1	0.3

Comparing it with previous statistics (1) the incidence of T. *violaceum* seems to diminish and *M. canis* seems to increase. The incidence of *T. schoenleinii* and *T. tonsurans* is similar to others registered before in the same region.

2. Tinea unguium. In 70 cases (including 29 with lesions in other sites) there were affected: a) finger-nails in 48; b) toe-nails in 17; and c) both finger and toe-nails in 5.

The following species were isolated:

a) Finger-nails (48 cases):

	number of cases	%
T. violaceum	20	41.7
T. megninii	15	31.2
T. rubrum	7	14.6
T. tonsurans	4	8.3
T. mentagrophytes	1	2.1
Scopulariopsis sp.	1	2.1

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[1]) All the 275 cases of Tinea capitis without lesions in other sites were observed in children.

b) Toe-nails (17 cases):

	number of cases	%
T. megninii	7	41.2
T. mentagrophytes	5	29.4
T. rubrum	2	11.7
T. tonsurans	1	5.9
E. floccosum	1	5.9
Candida albicans	1	5.9

c) Finger and toe-nails (5 cases):

	number of cases	%
T. rubrum	2	40.0
T. tonsurans	1	20.0
T. megninii	1	20.0
T. schoenleinii	1	20.0

A clear difference between some of the etiological agents in finger-nails and toe-nails was registered. *T. violaceum*, which was prevalent in finger-nails, did not appear in toe-nails. *T. mentagrophytes*, rather frequent in toe-nails, has only been detected once in finger-nails. The incidence of *T. megninii* and *T. rubrum* was nearly the same in both localizations.

3. Tinea corporis. In 82 cases (including 36 with lesions in other sites) were obtained:

	number of cases	%
T. tonsurans	18	21.9
T. megninii	17	20.7
T. violaceum	14	17.1
T. rubrum	12	14.6
M. canis	11	13.4
E. floccosum	6	7.3
T. mentagrophytes	4	4.9

T. tonsurans, T. megninii, T. violaceum and *T. rubrum* caused mainly chronic lesions and *M. canis, E. floccosum* and *T. mentagrophytes* were responsible for acute lesions.

4. Tinea barbae. In 23 cases (including 3 with lesions in other sites) were obtained:

	number of cases	%
T. megninii	13	56.5
T. tonsurans	4	17.4
T. violaceum	4	17.4
T. mentagrophytes	1	4.3
T. verrucosum	1	4.3

T. megninii was the prevalent agent of tinea barbae, followed by *T. tonsurans* and *T. violaceum*. The two cases by *T. mentagrophytes* showed acute and inflammatory lesions probably transmitted by animals.

5. Tinea cruris. In 42 cases (including 17 with lesions in other sites) were obtained:

	number of cases	%
E. floccosum	25	59.5
T. rubrum	13	30.9
T. megninii	2	4.8
T. mentagrophytes	1	2.4
Candida albicans	1	2.4

So there was registered a marked prevalence of *E. floccosum* and *T. rubrum* in tinea cruris.

6 Tinea pedis.

a) Soles. In 52 cases (including 15 with lesions in other sites) were obtained:

	number of cases	%
T. mentagrophytes	39	75.0
E. floccosum	8	15.4
T. rubrum	2	3.8
T. megninii	1	1.9
T. violaceum	1	1.9
M. canis	1	1.9

T. mentagrophytes was the most frequent agent in this localization. Figure 1 shows the distribution of 63 cases of tinea pedis where fungi were demonstrated (by direct examination, by culture or by both methods), related to the month when the disease was first observed. The great majority of cases appeared in summer; the decrease observed in August was probably related to vacations.

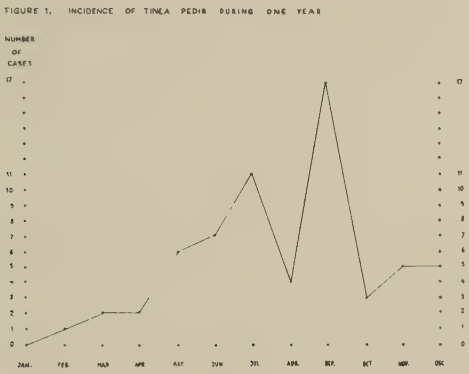

Fig. 1

b) Toe interspaces. In 30 cases (including 21 with lesions in other sites) were obtained:

	number of cases	%
T. mentagrophytes	16	53.3
E. floccosum	5	16.7
T. rubrum	4	13.3
T. megninii	4	13.3
Candida albicans	1	3.3

A prevalence of *T. mentagrophytes* was again noted. In all cases with lesions limited to the toe interspaces only *T. mentagrophytes* and *T. rubrum* were isolated; the other fungi appeared in cases with simultaneous lesions in other sites.

7. *Forms with multiple localization.* In 66 of 519 cases (12.7%) there were lesions simultaneously in more than one localization. Table VI specifies the affected regions and the fungi obtained from them. Each localization was affected in the following percentages:

	Cases with multiple localization	Percentage from 66 multiple forms	Percentage of multiple forms related to the total number of cases in each localization
Glabrous skin	37	56.1	45.1
Nails	32	48.5	45.7
Toe interspaces	24	36.4	80.0
Groins	20	30.3	47.6
Soles	17	25.7	32.7
Scalp	8	12.1	2.8
Beard	3	4.5	13.0

It was verified that forms with multiple localizations are rather frequent and that any classical localization of tinea infections may co-exist with lesions in other sites.

The species of dermatophytes most frequent in Portugal, with the exception of *M. canis* and *T. schoenleinii* were obtained from these multiple forms. However, lesions of the glabrous skin in children with tinea capitis caused by *M. canis* were frequently seen, although mycological study was not performed.

In 55 of the 66 cases with multiple lesions the same agent was obtained from all localizations. In the remaining 11 cases direct examination was positive in every localization, but culturing was only positive from one of them. Not more than one species of dermatophytes was isolated from any case.

The causative agents varied with the localizations affected (Table VI). In nails and glabrous skin (15 cases) *T. megninii* (5 cases), *T. rubrum* (4 cases), *T. violaceum* (3 cases) and *T. tonsurans* (3 cases) were obtained. *T. violaceum* (6 cases) and *T. tonsurans* (2 cases) were isolated from simultaneous infections of the scalp, nails and/or the glabrous skin (8 cases). In tinea barbae associated with lesions of the glabrous skin and nails (3 cases), *T. tonsurans* (2 cases) and *T. megninii* (1 case) were present. *E. floccosum* (12 cases), *T. rubrum* (4 cases), *T. megninii* (3 cases) and *T. mentagrophytes* (1 case) caused tinea cruris associated with lesions of the glabrous skin, soles and toe interspaces (20 cases). Lesions on feet (soles or toe interspaces), associated with lesions in nails or in glabrous skin (20 cases) were infected by *T. mentagrophytes* (10 cases), *T. rubrum* (5 cases), *T. megninii* (4 cases) and *Candida albicans* (1 case). These results agree with the prevalence of *T. megninii*, *T. rubrum*, *T. tonsurans* and *T. violaceum* in tinea corporis and tinea unguium, *T. violaceum* and *T. tonsurans* in tinea capitis, *T. tonsurans* and *T. megninii* in tinea barbae, *E. floccosum* in tinea cruris and *T. mentagrophytes* and *T. rubrum* in tinea pedis.

DISCUSSION

The number of cases studied during 1959 does not allow any generalization about the etiology of tinea infections in Portugal. So, further investigation must be done. However, this work reveals some interesting features.

T. megninii seems to be more frequent in Portugal than in other countries, as is indicated by previous work (6). *T. verrucosum* was identified for the first time in Portugal. It suggests the possibility of isolating this dermatophyte from cases of animal ringworm in this country.

Tinea unguium and tinea corporis are caused by the same agents (*T. violaceum*, *T. tonsurans*, *T. megninii* and *T. rubrum*) and both localizations are frequently co-existing. Some chronic cases of tinea corporis and tinea unguium may also be associated with lesions of the scalp. However, *T. megninii* and *T. rubrum* are quite rare in the last region; this suggests that the scalp is particularly resistent to the invasion by these both red-pigment producing dermatophytes. Tinea barbae is mainly produced by *T. megninii, T. tonsurans* and *T. violaceum* and occasionally by *T. mentagrophytes* and *T. verrucosum* contracted from animals. Tinea cruris is generally due to *E. floccosum* and co-exists frequently with lesions in other sites of the glabrous skin. Most cases of tinea pedis are caused by *T. mentagrophytes* and frequently there are simultaneously lesions of the soles, toe interspaces and toe-nails.

The important percentage of cases with multiple localization enhances the unitary concept of tinea as the infection produced by dermatophytes in skin and its appendages independently of its clinical form or localization. The clinical distinction between the several classical forms is not affected by the unitary concept of tinea. This one seems important for the pathogenic understanding of the infection and has epidemiological value. In fact, the chronic forms are probably the main reservoir of parasites(14). It seems that some fungi prefer certain localizations. Thus, *T. violaceum* and *T. tonsurans* are frequent in the scalp, nails, glabrous skin and beard. *T. megninii* infects mainly the beard, glabrous skin and nails. *T. rubrum* invades the glabrous skin, nails and sometimes, the feet and groins. *T. schoenleinii* appears in the scalp but may also invade nails and the glabrous skin. *M. canis* is a rather frequent agent of tinea capitis in children and sometimes produces lesions in the glabrous skin, associated or not with lesions in the scalp. *E. floccosum* is very frequent in the groins, co-existing often in other sites of the glabrous skin; it may also invade exclusively the feet. This last localization is prefered by *T. mentagrophytes* in the great majority of the cases.

SUMMARY

The A. studied 572 cases of dematomycosis and identified the causative agent in 519 (90.7%). "Mosaic fungi" were observed through direct examination only in scales from feet, independently of demonstration of dermatophytes; for this reason diagnostic value was not attached to those figures. The nutritional tests in 126 strains confirmed GEORG's results and was particurlarly useful for the identification of *T. tonsurans, T. megninii* and *T. verrucosum.*

Account is given about the agents obtained from the different localizations. *T. verrucosum* was identified for the first time in Portugal, and *T. megninii* seemed more frequent than in other countries.

The A. found 66 cases with multiple localizations and assumes that tinea must be considered a single infection produced by dermatophytes in skin and its appendages independently of its clinical form or localization.

ACKNOWLEDGEMENTS

The A. is much indebted to Professor Juvenal Esteves, director of the departments of dermatology in the Hospital Curry Cabral and the Faculty of Medicine of Lisbon, to Dr. N. van Uden and Dr. Lídia do Carmo-Sousa from the department of Microbiology, Faculty of Sciences, Lisbon, for suggestions and work facilities and to Miss Maria Manuela Figueiredo Matias and Mr. J. Silva Custódio for technical assistance.

References

1. Neves, H. & Carmo-Sousa, L. do — "Medical and Veterinary Mycology in Portugal for 1949—1959", Mycopathol. Mycol. Appl. XIII, **2**: 135 (1960).
2. Oliveira, H. — "Tinea capitis. Contribuição para o seu estudo etiológico no centro de Portugal". Amatus Lusitanus, **8**: 19, 1950.
3. —,— — "Contribuição para o estudo dos dermatófitos portugueses. I — Dermatófitos do centro de Portugal", Trab. Soc. Port. Derm. Ven., **10**: 147, 1952.
4. — & Sá, M. M. — "Contribuição para o estudo dos dermatófitos portugueses. II — A microsporia no centro de Portugal (Estudo de 111 casos)", Arq. Inst. Patolog. Geral, Coimbra,) **6**: 1950—1952.
5. —,— — "Contribuição para o estudo dos dermatófitos portugueses. III — Dez casos de tinea capitis de etiologia mista observados no centro de Portugal", Arq. Inst. Patolog. Geral, Coimbra, **6**: 1, 1950—1952.
6. —,— —"Contribuição para o estudo dos dermatófitos portugueses. IV — —Tinea barbae", Trab. Soc. Port. Derm. Ven., **12**: 95, 1954.
7. —,— —"Endemia portuguesa de tinha. Suas características etiológicas", Act. Dermosifilig., **8**: 1954.
8. — & Evaristo, A. J. — "Contribuição para o estudo dos dermatófitos portugueses. V — A primeira estirpe de *Microsporum gypseum* identificada em Portugal", Coimbra Méd., **4**: 345, 1957.
9. —,— — "Contribuição para o estudo dos dermatófitos portugueses. VI — Etiologia da tinea capitis no centro de Portugal", Coimbra Méd., **4**: 541, 1958.
10. Georg, L. K. — "Use of a cycloheximide medium for the isolation of dermatophytes from clinical materials", Arch. Derm. and Syph., **67**: 355—361, 1953.
11. Conant, N. & al. — "Manual of Clinical Mycology", Saunders Company, Philadelphia, 1954.
12. Georg, L. K. — "Dermatophytes. New methods in classification", U. S. Department of Health, Education and Welfare, Public Health Service, Communicable Disease Center, Atlanta, Georgia, 1957; and — "Routine Nutritional tests for the identification of Dermatophytes", J. Bact., **74**: ~~113~~—121, 1957. 3(
13. Neves, H. — "*Trichophyton megninii* Blanchard, 1896. Its incidence and identification by nutritional tests", in publication in Dermatologica.
14. Esteves, J. — "Sur l'épidémiologie de la teigne du cuir chevelu au Portugal", Ann. Derm. et Syph., **86**(4): 379—382, 1959.

MOLD FLORA OF HUMAN SCALP HAIR

BY

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(25.III.1960)

It has been shown by FUENTES (1954), GEORG (1956) and others that dermatophytes may be isolated from the coats of apparently healthy animals. It was deemed of interest therefore to examine random samples of human scalp hair for the presence of dermatophytes or other keratinolytic fungi.

Hair samples were collected by enlisting the aid of eight local barbers. They were supplied with envelopes to collect hairs from the shoulder towel following each hair cut. Hairs from each sample were sprinkled on plates of a salt-agar medium containing: triple salt solution 10 ml, agar 20 g, and chloromycetin 50 mg, per litre. The triple salt solution contained 1% each of NaCl, $(NH_4)_2SO_4$ and K_2HPO_3. The plates were incubated at 25 ° C for one week and examined for growth under the stereoscopic microscope. Slow growing colonies and colonies which did not readily produce spores on this medium were transferred to a modified Sabouraud medium (Dextrose 40 g, BBL Phytone 10 g, Difco yeast extract 5 g, agar 20 g, streptomycin 30 mg, and chloromycetin 50 mg, per litre). Hairs enveloped by fungus growth were mounted and examined microscopically for disintegration or penetration by hyphae.

Ninety-seven samples of hair were examined. *Penicillium* spp. grew from 35 samples, *Cladosporium herbarum* from 18, Sphaeropsidales from 14, *Paecilomyces elegans* from 11 (all from the same barber shop), *Aspergillus* spp. from 10, *Alternaria* sp. from 7, *Cephalosporium* sp. from 6, and *Chrysosporium pannorum*, *Pullularia pullulans*, *Stemphylium* spp. and *Scopulariopsis brevicaulis* from 4. In addition, a variety of other common molds were encountered as occasional isolates. There was no growth from 18 of the samples. No dermatophytes were isolated, nor did any of the fungi penetrate or digest the hairs.

SUMMARY

Samples of human scalp hair were collected in barber shops. Cul-

tures from 97 samples yielded a variety of common molds, but no dermatophytes or other keratinolytic fungi.

BIBLIOGRAPHY

FUENTES, C. A., Z. E. BOSCH & C. C. BOUDET (1954), Occurrence of *Trichophyton mentagrophytes* and *Microsporum gypseum* on hairs of healthy cats. J. Invest. Dermat., **23**:311—313.

GEORG, L. K., E. A. HAND & R. A. MENGES (1956), Observations on rural and urban ringworm. J. Invest. Dermat., **27**: 335—353.

MEDICAL AND VETERINARY MYCOLOGY IN PORTUGAL FOR 1949-1959

by

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(8.II.1960)

Most part of the Portuguese mycological literature is concerned with ringworm infections, which constitute an important sanitary problem in this country. The other cutaneous and visceral mycoses, with the exception of aspergillosis, are rare in Portugal.

There are some previous surveys on this subject. Carneiro (11, 12) reviewed the literature published before 1948, Pinto-Lopes et al. (113, 114) did so with the papers published between 1950 and 1955 and one of us (94) reviewed the portuguese literature on cutaneous mycoses which appeared between 1948 and 1958.

I. MEDICAL MYCOLOGY

A. Cutaneous mycoses

Esteves (30, 33), Fonseca (51, 59) and Leitão (70) discussed, for the benefit of general practitioners, the clinical and sanitary aspects of tinea capitis and the fundamentals of its prophylaxis and treatment (32, 34, 54).

There is a number of publications (2, 15, 35, 40, 52, 53, 55, 56, 57, 58, 76, 86, 96, 97, 98, 101, 103, 107, 108, 109, 110, 111, 112, 122, 123, 128, 129, 133) about the epidemiology and etiology of tinea capitis. Table I sums up the work done on etiological aspects of tinea capitis in Portugal. *Trichophyton violaceum* is the commonest agent (60% of 5,172 cases); *T. tonsurans* (14%), *Microsporon canis* (10%) and *T. schoenleinii* (15%) are less frequent while *T. rubrum*, *T. megninii*, *T. mentagrophytes* and *M. audouinii* are very rarely found in scalp ringworm. The few cases by *M. audouinii* seem to have originated in foreign children (52). Favus of the scalp is more frequent in the north of Portugal, where social and economic conditions are less developed (52). Oliveira (101, 103) stated that the Portuguese dermatophytic flora is of the mediterranean type, tricho-

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[1]) Supported by a grant of the ,,Instituto de Alta Cultura'' Lisbon.

TABLE I

Author	Region	Date	Number of cases	Agents %							
				T. *violaceum*	T. *tonsurans*	T. *rubrum*	T. *megninii*	T. *mentagrophytes*	M. *canis*	M. *audouinii*	T. *schoenleinii*
S. Leite (76)	Algarve (South)	1951	104	62.5	30.8	—	—	—	0.9	—	5.8
M. Antunes (2)	Center and South	1953	680	59.9	15.7	0.9	—	0.4	16.7	—	6.4
A. Fonseca (52)	North	1953	1059	48.6	9.8	—	—	0.4	8.2	0.2	32.8
J. Ribeiro (123)	Algarve (South)	1953	577	72.8	22.4	—	0.3	—	0.9		3.6
J. Maia (86)	Porto (North)	1953	103	47.6	8.7	—	—	—	22.3	—	21.4
S. Rego (122)	Leiria (Center)	1955	92	69.5	26.1	—	—	—	2.2	—	2.2
A. Fonseca (57)	Ponte de Lima (North)	1957	435	77.5	16.2	1.3	—	—	0.9	—	4.1
A. Fonseca (58)	Porto (North)	1957	172	59.3	24.4	—	—	—	8.7	—	7.6
H. Oliveira (103)	Center	1950-58	1500	61.7	10.1	—	1.0	0.2	16.9	0.1	10.0
Total			5172	60.03	13.77	0.21	0.35	0.19	10.48	0.08	14.89

phytia being more frequent than microsporia and favus and *T. violaceum* being the prevalent agent. SAMPAIO (132) reported one case of tinea capitis due to *T. violaceum*, with the clinical aspect of favus and suggested the maintenance of the classical *Favotrichophyton* group of dermatophytes.

Some work has also been done on the etiology of tinea capitis in some overseas Portuguese territories. In Madeira (131) *T. tonsurans* seems to be the principal agent and in the Azores (126) out of 680 cases, 67% of *T. violaceum* and 21% of *T. schoenleinii* were obtained. In Portuguese West Africa (73) *T. ferrugineum* (96%) and *M. canis* (4%) were found. In Cabo Verde (89, 90, 91) *M. canis* was obtained from 88% and *T. ferrugineum* from 10% out of 131 cases.

Since 1949, 61 cases of tinea capitis by double infection have been reported (3, 52, 55, 57, 99, 123, 130, 132, 139,); *T. violaceum* was present in almost all these cases.

There is a general opinion that tinea capitis in Portugal is an endemic disease of childhood; microsporia is more frequent in early childhood and trichophytia occurs specially in the school age. There are, indeed, some references of tinea capitis in adults, particularly women (4, 13). ESTEVES et al. (43) observed 1% of adults among 3,191 cases of tinea capitis and FONSECA et al. (60) observed that in a total of 3, 948 cases of scalp ringworm the incidence of adults was 3% in trichophytia and 12% in favus. ESTEVES (40, 44)analyzed the epidemiology of tinea capitis in Portugal and stressed that some cases do not heal spontaneously after puberty, admitting that chronic cases in the adults, frequently associated with onychomycosis, seem to constitute an important reservoir of parasites. Treatment of tinea capitis has received special attention. TANISSA reviewed the topical treatment of the disease (137) and studied „in vitro" the anti-mycotic activity of cod-liver-oil and derivates (138). LAPA (68) treated 400 children with thallium acetate, obtaining 92% cures and reporting 5.4% toxic manifestations; only one child had serious complications, which disappeared completely after treatment with sodium thiomalate and ascorbic acid for one week. The same author (69) essayed sodium thiomalate on experimental acute intoxication by thallium acetate, in guinea-pigs. FURTADO (63) reported one case of encephalitis by thallium which recovered after treatment with "B.A.L."

There is a number of publications by itinerant teams from the Department of Health (52, 53, 55, 56, 57, 60, 122, 123, 127, 128, 129, 133,) and some dermatological hospitals (43, 107—112) in which the prophylaxis and treatment of tinea capitis are described. Epilation by X rays gave generally good results, but the necessity of later careful local treatment during some months was stressed. BRANDÃO (7) described the usual technique of X ray epilation and reported the results obtained with this method in 955 patients. He pointed out the necessity of sanitary control of children treated as out patients, because 17% of the patients did not wait until confirmation of cure. The author registered 8% of relapses which were mainly attributed to imperfect topical measures after irradiation or to reinfection. Some technical modifications of the method used in children younger than 2 years, or with nervous or mental disturbances or with abnormal configuration of the scull have also been described. Later, ESTEVES et al. (43) referred 95% cures in 3,000 patients treated during thirteen years with X ray epilation and FONSECA et al. (60) obtained 91% cures in 3,948 cases. LEITE et al. (74) studied the effects of X rays on the etiological agents of tinea capitis, using the same conditions utilized in epilation with doses from 400 to 2,000 r. The irradiated cultures did not show any macro- or microscopical alteration and conserved their growing and pigment producing capacity.

ESTEVES (32) stressed the necessity of selection of cases both from the etiological and clinical point of view as well as of extreme carefullness during treatment to obtain good results with any substance used in the local treatment of tinea capitis. The same author (32) and SAMPAIO (134) verified that microsporia cures more easily with local treatment than trichophytia, which generally needs previous epilation. SOBRAL & MANUELA ANTUNES (136) reviewed the methods employed in topical treatment of tinea capitis. In 1951, FERREIRA et al. (49) published a preliminary note on the successful local treatment of ringworm with a new fungistatic ("Lusomycin") obtained from *Rhodotorula glutinis* var. *lusitanica;* so far, no confirmation of their results has been published.

Salicylanilide and its derivates were essayed with good results by ESTEVES and MANUELA ANTUNES (31, 38), who described morphological alterations in parasitized hairs during the treatment (36, 37). There are also several studies of this substance "in vitro" by MATA & LUISA NUNES (87, 88) and MANUELA ANTUNES (5, 6).

Recently, ESTEVES & NEVES (47, 48) essayed the new antibiotic griseofulvin in the treatment of different forms of ringworm. After oral administration of 0.5 to 1 gram daily during 3 to 6 weeks they obtained cure in 11 children with tinea capitis caused by *T. violaceum*, *T. tonsurans* and *M. canis* and in 4 cases of favus of the scalp (*T. schoenleinii*) and referred to the probable reinfection in 4 cases of tinea capitis by *T. violaceum*. In 7 adults with chronic forms of tinea with multiple localizations treated with 1 to 4 grams daily, they verified: a) in the scalp lesions, cure after 3 to 6 weeks; b) in the nails, clear improvement or cure of superficial lesions and little modification of destructive lesions after five months; c) on the glabrous skin, there was in 4 to 5 weeks clearing or disappearance of lesions, which, however, relapsed regularly when treatment was interrupted. They also verified that griseofulvin did not prevent or modify the evolution of experimental dermatomycosis in some treated patients. They concluded that griseofulvin is a fundamental progress in the treatment of dermatomycoses but does not modify the individual factors responsible for the chronicity of tinea infection in adults.

There is little information about ringworm localized outside the scalp. OLIVEIRA (100) obtained, from 50 cultures of tinea barbae, *T. roseum* (*T. megninii*) in 90%, *T. cerebriforme* (*T. tonsurans*) in 6% and *T. violaceum* in 4%, and, in 9 cases of tinea corporis (97), *T. violaceum* in 7, *T. roseum* in 1 and *T. schoenleinii* in 1. The same author reported with EVARISTO (102) the first strain of *Microsporon gypseum* isolated in Portugal, from ringworm on the face of a child. ESTEVES et al. (43) referred to 5 cultures from nails (*T. violaceum* in 2 cases, *T. rubrum* in 2 cases and *T. mentagrophytes* in 1 case), 3 from the beard (*T. mentagrophytes* in 2 and *T. violaceum* in 1) and 15 from the glabrous skin (*T. violaceum* in 7, *T. rubrum* in 6, *T. mentagrophytes* in 5, *T. tonsurans* in 3, *T. megninii* in 1 and *M. canis* in 1).

LEITÃO (70) observed among 366 cases of dermatomycosis diagnosed between 1950—1951, 84.7% of tinea capitis, 4.4% of favus of the scalp, 0.8% of tinea barbae, 0.3% of kerion barbae, 4% of tinea corporis, 1% of trichomycosis, 3.9% of pityriasis versicolor and 0.9% of mycotic intertrigo. In a recent recapitulation of the dermatomycologic activity developed during thirteen years in the Department of Dermatology of Curry Cabral Hospital (Lisbon), ESTEVES et al. (43) reported 3,716 cases of dermatomycosis, which correspond to 15% of all the patients observed. The frequency of the various localizations was: scalp - 86%; glabrous skin — 4%; nails —4%; groins — 2%; feet — 2%; and beard — 2%. They confirmed the prevalence of *T. violaceum* in tinea capitis and stressed the importance of tinea capitis in adults (1%) and chronic forms with multiple localization (generally with onychomycosis) as reservoirs of the disease. Tinea capitis of the adult and onychomycosis were much more observed in females whereas tinea cruris was almost exclusively seen in males. The authors promoted sanitary education among the population of the surroundings of Lisbon, where they found tinea capitis in 3% of the children.

CASTELLANI (17, 18, 21, 22, 23, 24) and CASTELLANI & SILVA (25) published a series of papers dealing with dermatophytoses "tinea nigra" and other little known cutaneous mycoses, specially of tropical origin.

VAN UDEN (142) described a new slide culture technique and studied the anastomoses between several strains of *Microsporon*. With this technique anastomoses were observed between strains labelled *M. felineum*, *M. lanosum* and *M. canis* whereas no anastomoses were seen between strains of *M. langeroni* and *M. audouinii*. MANUELA ANTUNES (1) did not notice any advantage on direct examination of parasitized hairs with contrasting phase microscope, as compared with normal microscope. RÉ (120) studied cultures of pigmented *Trichophyton* in a brain medium. Some strains recovered their pigment producing capacity, which had previously been lost in laboratory; in some cases pleomorphism appeared easly. LEITE & RÉ (79) reviewed the pleomorphism of dermatophytes and tried to avoid it using cultures with soil medium. LEITE & MANUELA ANTUNES (81, 82) maintained, with liophylization, the morphological and biological characteristics of dermatophytes for more than one year.

NEVES (93) reported on a study of experimental dermatomycosis in man. Experimental lesions with *T. violaceum*, similar to natural ringworm, were limited to the inoculated area and always healed spontaneously between eleven to eighty days. It seemed to exist individual susceptibility to this infection, because part of the individuals inoculated did not develop lesions. Later, ESTEVES & NEVES (42, 46) compared experimental infection with *T. violaceum* in patients with and without previous dermatomycosis. It seemed that previous infection shortened the time of incubation, and the size and duration of the experimental lesions; no differences were observed with regard to trichophytin tests done before and after inoculation. The same authors (45) studied two cases of kerion barbae caused by *T. mentagrophytes* of animal origin, with strongly positive trichophytin reactions, even with generalized hypersensitivity in one case. The inoculation of those strains in 9 volunteers always originated experimental lesions on glabrous skin and strong reactivity to trichophytin.

There are very few reports of deep cutaneous mycoses in Portugal. LEITE (72)reported on the existence of sporotrichosis in Portuguese West Africa and published a list of the fungi which are kept in culture in the Institute for Tropical Medicine (Lisbon)(77). COSTA (28) studied the pathology of some cases of deep cutaneous mycoses and ESTEVES & MANUELA ANTUNES (39) observed a granulomatous mycosis in a patient from Portuguese Guinea, from which CASTELLANI isolated a strain of *Aleurisma castellanii*. CASTELLANI et al. (26) proposed the creation of a new genus of actinomycete — *Actinomonospora* — to include a new species *A. lusitanica* isolated from a mycetoma and characterized by the production of a non difusible pigment, absence of aerial mycelium, reproduction by terminal conidia arising singly from indiferenciated and generally long branches.

CARRASCO et al. (14) reported on the second and third autochtonous cases of mycetoma in Portugal.

OLIVEIRA & TRINCÃO (104) studied and discussed the etiology of a case of black tongue, from which cultures of *Candida krusei* were obtained.

B. Visceral mycoses

Visceral mycoses have rarely been reported from Portugal.

GUIMARÃES (65) emphasized the clinical polymorphism of systemic mycoses and the difficulties of their diagnosis. CASTELLANI (16, 19, 20) registered in Portugal the occurrence of bronchomycoses simulating pulmonary tuberculosis and tonsillomycoses simulating diphtheria and follicular tonsilitis. NORONHA (95) reviewed the clinical aspects of actinomycosis. GARÇÃO (64) described one case of pleuro-pulmonary actinomycosis and PADESCA (106) reported on two more cases, calling attention to the occurrence of pulmonary mycoses in Portugal.

CAMPOS (8,9) reported on two cases of mycotic meningo-encephalitis, one probably caused by a *Candida* and the other by *Torulopsis neoformans*; both cases were diagnosed by autopsy.

HORTA et al. (66, 67) reported on the second portuguese case of histoplasmosis, diagnosed by bronchoscopy; the patient had lived some years in Brasil and suffered from skin lesions and respiratory symptoms similar to those of tuberculosis. LEITE obtained cultures of *Histoplasma capsulatum* from sputum and cutaneous exsudate of this patient. VAN UDEN et al. (144) carried out histoplasmin skin tests in 955 individuals from different parts of Portugal and found only 28 positive reactions which became negative or doubtful after retesting; complement fixation reactions were also negative in these individuals. They concluded that no widespread histoplasmin sensitivity exists in this country and, further, no definitive conclusions should be drawn from isolated weakly positive reactions. They admitted, however, that a few small foci of histoplasmosis may exist in Portugal and suggested methods for their detection. VAN UDEN et al. (145) studied also histoplasmin sensitivity in 81 individuals from Portuguese Africa, Goa and Macau, living then in Portugal and found only 2 positive reactors, who had lived in Luanda (Portuguese West Africa).

RÉ (121) studied the serological relationship between *Blastomyces dermatitides*, *B. brasiliensis* and *Histoplasma capsulatum*. A narrow relationship between the two species of *Blastomyces* was observed. Between these and *H. capsulatum* the relationship was not so evident. RODRIGUES (124, 125) reported on two cases of broncho-pulmonary aspergillosis by *Aspergillus fumigatus*; in both cases the fungus developed inside a cavity, which was a sequel of tuberculosis treated with chemotherapeutic agents. The author admits that broncho-pulmonary aspergillosis will not be such a rarely diagnosed condition when its clinical and radiological pictures will be better known. Later, PINTO et al. (119) referred to two more cases and VILLAR (158) presented other three cases of aspergilloma of the lung. PIMENTEL (116, 117, 118) described the macro- and microscopic morphological aspects observed in 15 cases of pulmonary aspergilloma and expressed his opinion about its pathogenesis: the fungus seems always to grow in pre-existing pulmonary cavities and provokes the formation of a foreign body granuloma.

LEITE et al. (84) reported on the conservation by liophylization of pathogenic fungi. The strains kept under such conditions during 2 years showed no modification of their morphological and pathogenic properties. The same authors (85) presented a new etiological classification of human mycoses which they assume to have a didactic value.

II. VETERINARY MYCOLOGY

A. Pathology

Van Uden (143) reported on the intraperitoneal inoculation of white mice with *Acladium castellanii*. After 3 weeks, 6 of the 10 animals inoculated showed nodules in the liver, spleen and small intestine. Morphology of the strain studied in lesions and in culture at 37ºC differs from *Blastomyces dermatitides* and *B. brasiliensis* by its filamentous aspect instead of a yeast-like one.

Frazão (61, 62) recorded the poisoning of bovines due to the ingestion of gramineous plants (*Paspalum distichum* L.) infected by *Claviceps paspali* in Sado's Valley.

According to Cruz (29) live stock ringworm and poultry pulmonary aspergillosis have been detected in Portugal.

Moreira-Jacob & Van Uden (92) described a case of bovine abortion probably caused by *Aspergillus niger* and reviewed the literature on bovine mycotic abortion, discussing various aspects of this disorder, e.g. its incidence, etiology and mode of infection.

Van Uden & Carmo-Sousa (153) isolated from the cecum of 252 bovines 125 *Aspergillus* strains belonging to the following species: *A. fumigatus*, *A. niger*, *A. terreus*, *A. nidulans*, *A. flavus*, *A. restrictus*, *A. glaucus*, var. *ruber* and *A. glaucus* var. *chevalieri*. From the cecum of 252 equines, 101 strains were isolated belonging to *A. fumigatus*, *A. niger*, *A. terreus*, *A. nidulans* and *A. flavus*. The ecological, epizootical, pathological and specially the toxicological significance of these findings was discussed.

Van Uden (143) described *Torulopsis pintolopesii* — a fastidious and thermophilic new yeast species isolated from the liver and spleen of 3 white mice inoculated with *Acladium castellanii*.

Van Uden et al. (148) isolated, from vegetable sources, 5 strains of *Candida albicans* which proved to be pathogenic for rabbits. The authors assumed that non-animal substrata though not a natural habitat for this yeast species, may probably support growth or at least permit its survival during considerable time, after having been contaminated from man or animals whose intestinal tract constitutes the natural habitat of *C. albicans*.

The same authors (147) studied the relationship between *Candida claussenii* and *C. albicans* through physiological, serological and pathological tests. It was concluded that those two yeast species are nearly related. The clinical and anatomo-pathological aspects of the experimental disease produced in rabbits, guinea pigs and mice by endovenous and subcutaneous injection of cells of *C. albicans* and *C. claussenii* were described.

The taxonomy, ecology and pathological significance of yeasts associated with the digestive tract of a number of different warm-blooded host species have been discussed in a series of papers from the Department of Microbiology, Botanical Institute, Lisbon:

a) VAN UDEN & CARMO-SOUSA (152) isolated 131 yeast strains belonging to 17 different species from the cecum of 252 adult bovines. *Candida albicans* was not isolated. They considered the apparent absence of *C. albicans* from the bovine intestinal tract as explaining the rarity of oral and intestinal moniliasis in bovines. Infection in certain forms of yeast mastitis (caused neither by *C. albicans* nor by *Cryptococcus neoformans*) is supposed to originate from the intestinal tract. Two new yeast species, *Pichia bovis* and *Candida bovina* were described.

b) The same authors (151) described *Candida slooffii* a new yeast species isolated from the cecal content of horses. Its fastidiousness and particularly its narrow temperature range (growth occurs only between 28° and 44°C) are considered characteristic for microorganisms adapted to life in warm-blooded hosts. Together with *Torulopsis pintolopesii* it was at the time the only known thermophilic yeast.

c) VAN UDEN et al. (156) studied the pathogenicity of a strain of *Cryptococcus neoformans* for mice isolated from the cecal contents of a horse. A detailed description of histopathological findings in experimental mouse cryptococcosis was given. A comparative study of some physiological properties of 23 strains of *C. neoformans* was made. *C. neoformans* was considered not probably to be a true intestinal saprophyte but as surviving for some time in the equine digestive tract. Equines may so constitute a vector in the dispersal of that yeast species. *C. neoformans* var. *innocuous* was considered a *nomen nudum* which does not correspond to a definite taxonomic entity but has been used to design any *Cryptococcus* sp. different from *C. neoformans*, found in association with man or animals.

d) VAN UDEN & ASSIS-LOPES (150) studied a new yeast variety isolated from the digestive tract of humans and a horse. It was named *Saccharomyces italicus* var. *melibiosi* and differs from the species by its ability to ferment and assimilate melibiose and the galactose part of raffinose. It was the first taxonomic yeast entity so far known to ferment raffinose without fermenting sucrose.

e) VAN UDEN et al. (154) isolated from the cecum of 252 horses, 503 sheep, 250 goats and 250 swine, 486 yeast strains belonging to 28 species and 1 variety. The distribution of the yeast of any species and of *C. albicans* respectively was: horses 52.4%, 4.4%; sheep 6.8%, 4.2% goats 6.4%, 0.8%; swine 88.8%, 9.2%. The suitability of sheep and goats as hosts for yeasts of any species seemed very limited. The most frequent occurrences for single species were: *Candida slooffii* in swine (48.4%), *Trichosporum cutaneum* in horses (21.8%) and *Saccharomyces tellustris* (*Candida bovina*) in swine (14%). Some physiological properties of *S. tellustris, Torulopsis glabrata, T. pintolopesii, Candida bovina, C. slooffii, Cryptococcus neoformans* and *Trichosporum cutaneum* were studied.

f) VAN UDEN & FARINHA (155) and VAN UDEN & CARMO-SOUSA (157) studying the significance of temperature relations and vitamin deficiency patterns in the delimitation of yeast species showed that in wild yeast species the intraspecific variation of these characteristics is of a very low order and that their inclusion in taxonomic work is justified and useful. They concluded respectively that:

1. *Candida zeylanoides* on account of its maximum temperature of growth (30— 32°C) is unable to grow in warm-blooded hosts while *C. norvegensis* nov. comb. may develop in this substrata.

2. Yeast strains which might be classified as *Candida parapsilosis* according to Lodder & Kreger-van Rij's system proved to belong to more than one species. An emended description of *C. parapsilosis* was given and a new species, *C. lusitaniae* was described. The problem was raised of knowing which of these entities has been responsible for the pathological cases so far recorded as caused by *C. parapsilosis*. Both species may develop in warm-blooded hosts. Every strain of *C. lusitaniae* so far studied came from the intestinal tract of warm-blooded animals. Furthermore, emended descriptions of *C. parapsilosis* var. *intermedia* and *C. obtusa* nov. comb. were given as well as the diagnosis of the new variety *C. parapsilosis* var. *querci*.

Cruz (29) diagnosed actinomycosis in live stock.

Petisca (115) described a case of generalized actinomycosis in a swine. Embolic foci were localized in the lungs, spleen, liver and mesenteric and mediastinal lymph nodes. Primary localization was supposed to be in the mandibulas lymph nodes.

B. Technology

In a paper dealing with the problems of sausage inspection Paiva (105) included a review on the mycological flora so far recorded in such meat products.

Tropa (140) studying the "dim" in codfish showed its etiology to be due to *Sporendonema epizoum* (Corda) Cif. et Red.

Castro (27) pointed out that "dim" could be discarded if codfish was well washed with water and treated afterwards with a 4% solution of sodium metabisulphite. He gave no information about the toxicity of such product to human health.

The experiments carried out by Tropa et al. (141) showed that potassium metabisulphite is toxic for guinea-pigs and dogs. It strongly suggests the necessity of avoiding the use of metabisulphite as food perservative.

Geotrichum candidum has been found in yogurt as contaminant by Carmo-Sousa & Van Uden. (10)

Van Uden & Assis-Lopes (146) working with kefir grains received from various countries, isolated the yeasts present in them in order to determine which should be selected for the industrial production of this dietetic fermented milk. It was concluded that *Saccharomyces delbruckii* is the most suitable species.

Van Uden & Carmo-Sousa (149) stated that yogurt may be heavily infected with lactose fermenting yeast, e.g. *Candida pseudotropicalis*.

They found that coliform free yogurt produced in the laboratory containing 6,850,000 or more cells of a strain of *C. pseudotropicalis* per ml. may give false positive reactions for coliform organisms with brilliant green lactose peptone bile broth. * With formate ricinoleate lactose peptone broth*, however, no false positive reactions were obtained with as much as 340,000,000 cells of *C. pseudotropicalis* per ml. of inoculated yogurt.

Carmo-Sousa & Van Uden (10) analyzed yogurt produced in Lisbon and noted its contamination by yeasts of the genus *Candida*. *Candida pseudotropicalis* was the most frequent and the most important contaminant. It was found in 96 out of 136 samples mostly in numbers between 10,000,000 and 60,000,000 cells per ml. This yeast species causes an alcoholic fermentation which changes the organoleptic characteristics of yogurt. Heavy contamination of this product by fermentative yeasts may be dangerous for debilitating people originating or agravating intestinal dispepsia. Experimental work permitted to establish the rules of avoiding or reducing to sporadic cases, the contamination by yeasts. Practical indications for mycological analyzis of yogurt were given.

Santos (135) stated that yeasts are present in the rind of "Serra" cheese in numbers which may reach some thousands per gram. They decrease in the subjacent layers and are pratically absent in the depth. Yeasts are abundant in the superficial viscosity developed during the early ripening period. He supposes that the ripening is produced by enzymes of the bacterial flora of the rind in spite of the proteolytic activity shown by some yeast strains isolated from "Serra" cheese. The yeasts induce pH modifications which might favor an increase of the bacterial growth.

References

1. Antunes, M. M.: ,,Aplicação da microscopia em contraste de fase ao diagnóstico laboratorial das tinhas". Trab. Soc. Port. Dermat. Ven., **9**(1): 48, 1951.
2. Antunes, M. M. :,,Agentes etiológicos da tinha do couro cabeludo na Estremadura e Sul de Portugal". Médico, **4**(111): 843, 1953 e Trab. Soc. Port. Dermat. Ven., **11**(3): 111, 1953.
3. Antunes, M. M.: ,,Tinhas tonsurantes de etiologia mista (Nota acerca de 4 casos)." Médico, **4** (115): 935, 1953 e Trab. Soc. Port. Dermat. Ven., **11** (3): 129, 1953.
4. Antunes, M. M.: ,,A tinha tonsurante depois da puberdade." Médico, **4** (116): 931, 1953.
5. Antunes, M. M.: ,,Acção *in vitro* do creme de Salicilanilida, do Asterol e do Furaspor sobre *Trichophyton violaceum*, *T. acuminatum* e *Microsporum felineum*". An. Azevedos, **5** (3): 154, 1953 e Trab. Soc. Port. Dermat. Ven., **11** (1): 15, 1953.
6. Antunes, M. M.: ,,Estudo da acção da Clorosalicilanilida sobre estirpes de *Trichophyton violaceum*, *T. acuminatum*, *T. rubrum* e *Microsporum felineum* e efeito terapêutico nas tinhas tonsurantes". An. Azevedos, **4** (4): 232, 1954.
7. Brandão, N.: ,,A roentgenterapia das tinhas do couro cabeludo". Médico, **4** (111): 857, 1953 e Trab. Soc. Port. Dermat. Ven., **12** (2): 103, 1954.

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*) Used according to the directions of the "Standard Methods for the Examination of Dairy Products" (1953) 10th edition. New York Amer. Public Health Ass. Inc.

8. Campos, J. O.: ,,Meningo-encephalite mycosique chez un enfant âgé de 7 jours". Rev. Clin. Inst. Maternal, **3** (8), 1951.
9. Campos, J. O.: ,,Preliminary report of congenital meningo-encephalitis due to *Torulopsis neoformans*". Bol. Clin. Hosp. Civ. Lisboa, **18:** 609—618, 1954.
10. Carmo-Sousa, L. do & Uden, N. van: ,,Estudo sobre a contaminação do iogurte com levedura". Bol. Pecuário, **26** (2): 5—27, 1958.
11. Carneiro, A. L.: ,,As micoses em Portugal (Revista crítica)". Publ. Inst. Botânico Faculd. Cienc. Universid. Porto, 1948.
12. Carneiro, A. L.: ,,Ten years of investigation about mycopathology in Portugal (1938—1948)". Mycopathol. Mycol. Appl. **5** (1): 91, 1950.
13. Carrasco, M. C.: ,,A tricofitia do coiro cabeludo do adulto (Apresentação de um caso com alopécia cicatricial)". Gaz. Méd. Port., **2** (1): 123, 1949.
14. Carrasco, M. C., Leite, A. S., Sobral, C. & Ré, L.: ,,Os segundo e terceiro casos portugueses autóctones de micetoma". Bol. Clin. Hosp. Civ. Lisboa, **20** (1—2): 231, 1956 e Gaz. Méd. Port., **9** (2): 169, 1956.
15. Castanheira, F. G.: ,,A tinha do coiro cabeludo na província do Algarve". Bol. Serv. Saúde Públ., **2** (2): 55, 1955.
16. Castellani, A.: ,,Bronchomycoses simulating pulmonary tuberculosis. Tonsillomycoses simulating diphteria and follicular tonsillitis. Occurrence of these affections in Portugal". XIII Cong. Luso-Espanhol para o progresso das Ciências, 9, 8a Sec.: 31—54, 1950.
17. Castellani, A.: Mycological studies on *Tinea nigra* and other little known mycoses (Cryptococcosis epidermica, Accladiosis, Paraccladiosis, Tinea tenuishypha, Tinea pedum geotrichia, Fissura mediana labialis blastomycetica)". Intern. Congr. Microbiology, 1950.
18. Castellani, A.: ,,Aspectos clínicos e frequências das micoses cutâneas e das Tricomicoses na velhice". J. Soc. Cienc. Méd. Lisboa, **115** (7): 179, 1951.
19. Castellani, A.: ,,Bronchomycosis simulating pulmonary tuberculosis. Occurrence of these affections in Portugal." Imp. Med., **16** (12): 587—605, 1952.
20. Castellani, A.: ,,Some notes on tonsillomycoses affections at times simulating diphteria and follicular tonsillitis". Imp. Med., **17** (1): 1—5, 1953.
21. Castellani, A.: ,,Further observations on some little known tropical and subtropical diseases, internal, surgical and cutaneous". An. Inst. Med. Trop. **10** (3): 1891—2182, 1953.
22. Castellani, A.: ,,Mycotic pruritus ani." Impr. Méd., **17** (2): 96, 1953.
23. Castellani, A.: ,,A brief note on the treatment of paronychia moniliaca (paronychia candidiaca), fissura interdigitalis moniliaca (erosio interdigitalis blastomycetica) and onychomycosis trichophytica". Impr. Med., **18** (5): 312—314, 1954.
24. Castellani, A.: ,,Superficial cutaneous mycoses and trichomycoses in old age." Geriatrics, **10** (2): 86—88, 1955.
25. Castellani, A. & Silva, R. de: ,,Tinea nigra." J. Trop. Med. Hyg., **60** (8): 3—8, 1957.
26. Castellani, A., Brito, M. M. X. de & Pinto, M. R.: ,,An actinomycete isolated from an autochtonous case of mycetoma in Portugal". J. Trop. Med. Hyg., **62** (2): 27—36, 1959.
27. Castro, J. O. de: ,,Breve modificação no processo prático de beneficiar o bacalhau ,,empoado". Rev. Ciências Vet., **57** (364): 98—110, 1958.
28. Costa, J. G.: ,,Alguns casos de micoses cutâneas". Trab. Soc. Port. Dermat. Ven., **13** (3): 175, 1955.
29. Cruz, A. A. da: ,,Les maladies parasitaires du bétail au Portugal". Bull. Off. Int. Epizooties, **43** (1—2): 269—282, 1955.
30. Esteves, J.: ,,A tinha". An. Azevedos, **3** (2): 66, 1951 e Médico, **2** (19): 193, 1951.
31. Esteves, J.: ,,O tratamento da tinha do coiro cabeludo pela Salicilanilida". J. do Méd., **18** (456): 645, 1951.
32. Esteves, J.: ,,A propósito do tratamento local da tinha do coiro cabeludo". An. Azevedos, **3** (5): 242, 1951.
33. Esteves, J.: ,,A tinha como flagelo médico-social da criança". I Congr. Nac. Protecção à Infância, Lisboa, Nov. 1952. Médico, **4** (70): 23, 1953.

34. Esteves, J.: „Organização e métodos na clínica e estudo da tinha". Médico, **4** (110): 826, 1953.
35. Esteves, J.: „Algumas características etiológicas da endemia portuguesa da tinha". Médico, **4** (110): 831, 1953 e Trab. Soc. Port. Dermat. Ven., **11** (4): 191, 1953.
36. Esteves, J. & Antunes, M. M.: „Alterações morfológicas dos cabelos parasitados observados no decurso do tratamento das tinhas tonsurantes do couro cabeludo pela Salicilanilida". An. Azevedos, **5** (5): 277, 1954 e Trab. Soc. Port. Dermat. Ven., **11** (3): 105, 1953.
37. Esteves, J. & Antunes, M. M.,: „Morphological alterations in parasitized hairs observed in the course of the topical treatment of tinea tonsurans of the scalp". Dermatologica **115** (4): 496, 1957.
38. Esteves, J. & Antunes, M. M.: „Acerca do tratamento local das tinhas tonsurantes com Salicilanilida". An. Azevedos, **6** (2): 91, 1954 e Trab. Soc. Port. Dermat. Ven., **12** (1): 11, 1954.
39. Esteves, J. & Antunes, M. M.: „Acladiosis (Report on a case)". Doc. Med. Geograph. Tropica, **7:** 140, 1955.
40. Esteves, J., Fonseca, A. & Antunes, M. M.: „Aspectos epidemiológicos da tinha em Portugal". Port. Méd., **39** (2): 65, 1955.
41. Esteves, J., Fonseca, A. & Antunes, M. M.: „Acromia parasitária devida ao *Microsporum felineum*". Trab. Soc. Port. Dermat. Ven., **15** (1): 43, 1957.
42. Esteves, J. & Neves, H.: „Estudo preliminar da infecção humana experimental pelo *Trichophyton violaceum*". VI Congr. Med. Tropical, Lisboa, Set. 1958. Abstracts of the papers, pg. 149.
43. Esteves, J., Brandão F. N., Neves, H. & Custódio, J. S.: „Ensaio de integração funcional de serviço hospitalar em actividade dermatómicológica", Bol. Clin. Hosp. Civ. Lisboa, **23:** 307—330, 1959.
44. Esteves, J.: „Sur l'épidémiologie de la teigne du cuir chevelu au Portugal" Ann. Derm. et Syph., **86:** 379—382, 1959.
45. Esteves, J. & Neves, H.: „Foliculites supuradas da barba provocadas por fungos (Kerion da barba)", J. do Méd., **38:** 785—793, 1959.
46. Esteves, J. & Neves, H.: „Vorläufige Untersuchung der experimentellen Infektion durch *Trichophyton violaceum* beim Menschen". Hautarzt, **10:** 151—154, 1959.
47. Esteves, J. & Neves, H.: „A griseofulvina no tratamento da tinha do coiro cabeludo e de outras localizações desta doença. Primeiras observações", J. do Méd., **39:** 341—344, 1959.
48. Esteves, J. & Neves, H.: „Griseofulvin. Therapeutic results in different dermatomycosis after 22 weeks of treatment. Effects on experimental dermatomycosis in man". Dermatologica, **119:** 148—158, 1959.
49. Ferreira, M., Carneiro, A. L., Pinto, M. R., Pereira, A. L. & Gomes, J. M: „Possibilidades de tratamento da tinha pela Lusomicina". Trab. Soc. Port. Dermat. Ven., **9** (3): 157, 1951.
50. Fonseca, A. & Menezes, L. Q.: „O tratamento das tinhas no Albergue de Mendicidade do Porto." J. do Méd., **14** (341): 153, 1949.
51. Fonseca, A. & Menezes, L. Q.: „Patologia e tratamento das tinhas". Médico, **2** (30): 475, 1951 e Rev. Port. Pediat. Puericult., **15** (2): 61, 1952.
52. Fonseca, A. & Menezes, L. Q.: „Aspecto epidemiológico da tinha no Norte de Portugal". Médico, **4** (110): 833, 1953 e Trab. Soc. Port. Dermat. Ven., **11** (4): 187. 1953.
53. Fonseca, A., Wilhelm, O. & Macedo, C.: „A tinha do coiro cabeludo no concelho de Póvoa do Varzim". Médico, **4** (110): 837, 1953 e Trab. Soc. Port. Dermat. Ven., **12** (1): 31, 1954.
54. Fonseca, A., Wilhelm, O. & Macedo, C.: „Esboço de organização da luta contra a tinha do coiro cabeludo no Norte de Portugal." Médico, **4** (111): 863, 1953 e Trab. Soc. Port. Dermat. Ven., **11** (4): 187, 1953.
55. Fonseca, A., Wilhelm, O. & Macedo, C.: „Estudo epidemiológico da tinha do coiro cabeludo no Norte de Portugal". Médico, **5** (165): 887; (166): 926; (167): 952 e (168): 973, 1954.

56. Fonseca, A. & Lisboa, M. S.: ,,A tinha do coiro cabeludo no concelho de Viana do Castelo". Médico, **6** (201): 683 e (202): 713, 1955.
57. Fonseca, A. & Lisboa, M. S.: ,,A tinha do coiro cabeludo no concelho de Ponte de Lima". Bol. Serv. Saúde Públ., **4** (2): 255, 1957.
58. Fonseca, A. & Macedo, C.: ,,Alguns aspectos epidemiológicos da tinha do coiro cabeludo na cidade do Porto". Médico, **8** (305): 37, 1957.
59. Fonseca, A. & Macedo, C.: ,,Alguns aspectos médico-sanitários da tinha do coiro cabeludo". Médico, **6** (308): 109, 1957.
60. Fonseca, A., Basto, M., Oswald, W. & Macedo, C.: ,, A acção do Dispensário Central de Higiene Social do Porto na luta contra a tinha (Quinquénio de 1953—1957). Alguns aspectos epidemiológicos. Tratamento e resultados". O Médico, 403, 1959.
61. Frazão, T. L.: ,,Algumas considerações sobre a embriaguez dos bovinos no vale do Sado". Bol. Pecuário, **23** (2): 29—42, 1955.
62. Frazão, T. L.: ,,A embriaguez dos bovinos no Vale do Sado". Bol. Pecuário, **24** (1): 63—65, 1956.
63. Furtado, D: ,,Encefalite pelo tálio. Tratamento com B.A.L." J. do Méd., **18** (444): 145, 1951.
64. Garção, A.: ,,Um caso de actinomicose pleuropulmonar", Clin. Hig. Hidrolog. **15:** 67—76, 1949.
65. Guimarães, J.: ,,Pleomorfismo do diagnóstico das micoses viscerais", J. do Méd. **25:** 377—378, 1954.
66. Horta, J. S., Villar, T. & Marques, G.: ,,Um caso de histoplasmose diagnosticada pelo exame histológico da biópsia broncoscópica", J. Soc. Cienc. Méd. Lisboa, **117**, (7), 1953.
67. Horta, J. S., Villar, T. & Marques, G.: ,,A case of Histoplasmosis in Portugal diagnosed by bronchoscopic biopsy", Doc. Med. Geogr. et Trop., **5:** 347, 1953.
68. Lapa, C.: ,,Tratamento de quatrocentos casos de tinha pelo acetato de tálio". Gaz. Méd. Port., **3** (4): 808, 1950.
69. Lapa, C.: ,,Acção do Detoxicant (3606 R. P.) na intoxicação experimental pelo acetato de tálio". Gaz. Méd. Port., **3** (4): 812, 1950.
70. Leitão, A.: ,,Micoses cutâneas. Clinica e terapêutica". J. do Méd., **20** (507): 595, 1952.
71. Leitão, A.: ,,Aspectos clínicos da tinha em Portugal". III Congr. Hisp.-Port. Dermat., Santander, Sept. 1954. Act. Dermo Sifil., **8:** 1954.
72. Leite, A. S.: ,,Aspectos da Micologia Médica em Angola". Jorn. Méd. Luso-Ital., Roma, Junho 1949. J. do Méd., **14** (343): 199, 1949.
73. Leite, A. S. & Luz, J. B.: ,,Estudos sobre os agentes causais de tinha em nativos africanos de raça negra". Act. II Congr. Luso-Espanh. Dermat., 249, Lisboa, 1950.
74. Leite, A. S., Ré, L. & Sobral, F. C.: ,,Acção dos Raios X sobre fungos agentes de tinhas humanas". An. Inst. Med. Trop., **8** (2): 161, 1951.
75. Leite, A. S. & Ré, L.: ,,O tratamento do pé de atleta". Gaz. Méd. Port., **10** (3): 287, 1951.
76. Leite, A. S., Ré, L. & Antunes, M. M.: ,,Champignons des teignes au sud du Portugal (Algarve)." Atti del III Congr. Intern. d'Igiene e Med. Mediterranea, Palermo, Magg. 1951.
77. Leite, A. S., Ré, L. & Antunes, M. M.: ,,Relação dos fungos existentes na micoteca em 1953". Inst. Med. Trop., Lisboa, 1953.
78. Leite, A. S., Luz, J. B. & Ré, L.: ,,Acromia parasitária (Jeanselme)". Bol. Clin. Estat. Hospit. Ultramar, **6** (2): 147, 1953.
79. Leite, A. S. & Ré, L.: ,,A transformação pleomórfica dos dermatófitos". Ann. Inst. Med. Trop., **11** (1): 139, 1944.
80. Leite, A. S. & Ré, L.: ,,Contribution à l'étude ethnologique des populations africaines". Arch. Inst. Pasteur d'Algérie, **33** (4): 344, 1955.
81. Leite, A. S. & Antunes, M. M.: ,,Conservação por liofilização dos fungos agentes etiológicos das tinhas". Act. Dermo Sifil., **46:** 446, 1955.
82. Leite, A. S. & Antunes, M. M.: ,,Conservation par lyophilisation des champignons agents étiologiques des teignes". Comptes Rendues des séances de la Soc. Biologie, **149:** 1813, 1955.

83. Leite, A. S. & Ré, L.: ,,As micoses humanas mais frequentes. I — O pé de atleta (eczema micótico dos pés)". An. Inst. Med. Trop., **14,** Supl. 3: 3, 1957.
84. Leite, A. S., Ré, L. & Sobral, F. C.: ,,A conservação dos fungos patogénicos pelo processo da congelação-secagem (liofilização)" Mem. III Congr. Ibero Latino Americano Derm., 153—156, Mexico (1956) 1959.
85. Leite, A. S., Ré, L. & Sobral F. C.: ,,Uma tentativa de classificação etiológica das micoses humanas". Mem. III Congr. Ibero Latino Americ. Derm.: 244—247, Mexico (1956) 1959.
86. Maia, J. C.: ,,Alguns aspectos de epidemiologia, profilaxia e combate da tinea capitis". Dissert. de Doutoramento. Fac. Med. do Porto, 1953.
87. Mata, G. & Nunes, M. L.: ,,Contribuição para o estudo químico-analítico da Salicilanilida". An. Azevedos, **3** (1): 21; (3): 145, 1951.
88. Mata, G. & Nunes, M. L.: ,,Ensaios de actividade fungistática com novos derivados de ácidos gordos e da Salicilanilida". An. Azevedos, **5** (1): 9, 1953.
89. Meira, M. V., Nogueira, J. P. & Simões, T. S.: ,,Tinhas humanas em Cabo Verde. I-Estudo etiológico de alguns casos das ilhas de Boa-Vista e de S. Nicolau" An. Inst. Med. Trop., **15:** 867—869, 1958.
90. Meira, M. V., Nogueira, J. P. & Coito, A. M.: ,,Tinhas humanas em Cabo Verde. II-Estudo etiológico de alguns casos da ilha de Santo Antão". An. Inst. Med. Trop., **15:** 871—872, 1958.
91. Meira, M. V., Sousa, H. T. & Coutinho, M. A.: ,,Tinhas humanas em Cabo Verde. III Agentes etiológicos da tinea capitis na ilha de S. Vicente". An. Inst. Med. Trop., **15:** 873—877, 1958.
92. Moreira-Jacob, M. & Uden, N. van: ,,Mycotic abortion in cattle". Brit. Vet. J., **112** (10): 453—469, 1956.
93. Neves, H.: ,,Infecção humana experimental pelo Trichophyton violaceum". Trab. Soc. Port. Dermat. Ven., **15** (3): 167, 1957.
94. Neves, H.: ,,Micoses cutâneas em Portugal. Revisão bibliográfica e crítica (1948—1958)". Gaz. Méd. Port., **12;** 242—250, 1959.
95. Noronha, R.: ,,Apontamentos sobre actinomicose", Clin. Contemp., **3:** 1654—1658, 1949.
96. Oliveira, H.: ,,Tinea capitis. Contribuição para o seu estudo etiológico no centro de Portugal". Amatus Lusitanus, **8:** 19, 1950.
97. Oliveira, H.: ,,Contribuição para o estudo dos dermatófitos portugueses. I-Dermatófitos do centro de Portugal". Clin. Hig. Hidrol., Set. 1952 e Trab. Soc. Port. Dermat. Ven., **10** (3): 147, 1952.
98. Oliveira, H. & Sá, M. M.: ,,Contribuição para o estudo dos dermatófitos portugueses. II–A microsporia no centro de Portugal. (Estudo de 111 casos)." Arq. Inst. Patol. Geral, Coimbra, **6:** 1, 1950—52.
99. Oliveira, H.: ,,Contribuição para o estudo dos dermatófitos portugueses. III–Dez casos de tinea capitis de etiologia mista observados no centro de Portugal". Arq. Inst. Patol. Geral, Coimbra, **6:** 1, 1950—52.
100. Oliveira, H.: ,,Contribuição para o estudo dos dermatófitos portugueses. IV — Tinea barbae." Trab. Soc. Port. Dermat. Ven., **12** (2): 95, 1954.
101. Oliveira, H.: ,,Endemia portuguesa de tinha. Suas característi cas etiológicas". III Congr. Hisp. Port. Dermat., Santander, Set. 1954. Act. Dermo Sifil., **8:** 1954.
102. Oliveira, H. & Evaristo, A. J.: ,,Contribuição para o estudo dos dermatófitos portugueses. V – A primeira estirpe de *Microsporum gypseum* identificada em Portugal". Coimbra Méd., **4** (4): 345, 1957.
103. Oliveira, H.: ,,Contribuição para o estudo dos dermatófitos portugueses. VI – Etiologia da tinea capitis no centro de Portugal". Coimbra Méd., **4** (6): 541, 1958.
104. Oliveira, H. & Trincão, R.: ,,Língua negra pilosa (Considerações a propósito de um caso)". Coimbra Méd., **4** (7): 623, 1958.
105. Paiva, O. C.: ,,Inspecção sanitária dos enchidos e das matérias primas usadas na sua preparação." Bol. Pecuário, **19** (1): 1—237. 1951.
106. Padesca, A.: ,,Micoses pulmonares", Rev. Univ. Coimbra, **17:** 3—22, 1950.

107. Penella, S. & Roda, J.: ,,Estatística das Tinhas. Ano de 1946''. Trab. Soc. Port. Dermat. Ven., **6** (1): 47, 1948.

108. Penella, S. & Roda, J.: ,,Estatística das Tinhas. Ano de 1947''. Trab. Soc. Port. Dermat. Ven., **6** (3): 197, 1948.

109. Penella, S. & Roda, J.: ,,Estatística da Consulta de Tinhas (1935—1949)''. Act. II Congr. Luso Espanh. Dermat., Lisboa, pg. 247, 1950.

110. Penella, S. & Roda, J.: ,,Estatística das Tinhas do ano de 1950''. Trab. Soc. Port. Dermat. Ven., **9** (1): 32, 1951.

111. Penella, S., Sampaio, M. & Sobral, C.: ,,Estatística da Consulta de Tinhas referente ao ano de 1951''. Trab. Soc. Port. Dermat. Ven., **10** (2): 103, 1952.

112. Penella, S. & Roda, J.: ,,Estatística da consulta das Tinhas referente aos anos de 1952—53''. Trab. Soc. Port. Dermat. Ven., **12** (3): 189, 1954.

113. Pinto-Lopes, J., Uden, N. van & Cabral, R. G.: ,,Mycological Abstracts 1950''. Dep. Micol. Faculd. Cienc. Lisboa 1951.

114. Pinto-Lopes, J. & Ré, L.: ,,Mycological Abstracts 1951''; idem 1952; idem 1953; idem 1954; idem 1955; Dep. Micol. Faculd. Cienc. Lisboa.

115. Petisca, J. L. N.: ,,Actinomicose generalizada em suíno''. Rev. Med. vet., **45** (334): 106—113, 1950.

116. Pimentel, J. C.: ,,Aspergilose pulmonar de forma tumoriforme (Aspergiloma pulmomar). Contribuição para o conhecimento do seu quadro anatomo-patológico e da sua patogénese'', Med. Contemp., **76:** 241 — 268. 1958.

117. Pimentel, J. C.: ,,Aspergilose pulmonar, de forma tumoriforme (Aspergiloma pulmonar). Estudo anátomo-patológico de quatro casos e discussão da sua patogenia''. Gaz. Méd. Port., **11:** 455—472, 1958.

118. Pimentel, J. C.: ,,Novos aspectos morfológicos do Aspergiloma pulmonar. Seus prováveis estádios iniciais e residuais''. Gaz. Méd. Port. **12:** 195—210, 1959.

119. Pinto, E., Gonzaga, R., Alexandrino, S. & Neves, M.: ,,Dois casos de aspergiloma pulmonar tratados por lobectomia''. O Médico, **9:** 232—249, 1958.

120. Ré. L., ,,Aspectos da cultura de espécies pigmentadas de fungos do género *Trichophyton* em meio de cérebro.'' Trab. Soc. Port. Dermat. Ven., **9** (1): 23, 1951.

121. Ré, L.: ,,Reacçõcs serológicas entre o *Blastomyces dermatitidis* e *B. brasiliensis* e o *Histoplasma capsulatum*''. Mem. III Congr. Ibero Latino Americ., 236—238, Mexico (1956), 1959.

122. Rego, A. S. ,,As tinhas tratadas nos concelhos de Leiria, Marinha Grande, Batalha e outros, durante o ano de 1955''. Bol. Serv. Saúde Públ., **2** (2): 47, 1955.

123. Ribeiro, J. P.: ,,Contribuição para o estudo do facies dermatofítico da província do Algarve''. J. do Méd., **25** (622): 837, 1954.

124. Rodrigues, F.: ,,Aspergilose broncopulmonar. Nota prévia''. J. do Méd., **35:**, 149—156, 1958.

125. Rodrigues, F.: ,,L'aspergillome, mycose des cavités broncho-pulmonaires'' J. Franç. Méd. et Chir. Toracique, **13:** 179—192, 1959.

126. Salta, A. L. & Antunes, M. M.: Frequência e agentes etiológicos das Tinhas nas ilhas de São Miguel e Santa Maria (Açores). Nota prévia.'' Médico, **4** (114): 925, 1953.

127. Salta, A. L.: ,,Acção epidemiológica na Tinha''. Trab. Soc. Port. Dermat. Ven., **15** (2): 105, 1957.

128. Salta, A. L.: ,,A Tinha no distrito de Viseu''. Bol. Serv. Saúde Públ., **4** (1): 59, 1957.

129. Salta, A. L., & Castanheira, F. G.: ,,A Tinha no concelho de Vila Nova de Ourém''. Bol. Serv. Saúde Públ. **4** (1): 111, 1957.

130. Sampaio, N.: ,,Polimorfismo Cultural, macroscópico, do Endothrix cerebriforme em meio de Sabouraud''. Act. do II Congr. Luso Espanh. Dermat. Lisboa, pg. 259, 1950.

131. Sampaio, N.: ,,Sobre as Tinhas da Ilha da Madeira''. Act. do II Congr. Luso Espanh. Dermat., Lisboa, pg. 263, 1950.

132. Sampaio, N.: ,,Para a história do *Trichophyton violaceum*". Trab. Soc. Port. Dermat. Ven., **11** (1): 3, 1953.

133. Sampaio, N. & Rego, S.: ,,Brigadas móveis na luta contra a Tinha". Trab. Soc. Port. Dermat. Ven,. **12** (4): 225, 1954.

134. Sampaio, N. & Rego, S.: ,,Treze casos de tonsurante microspórica tratados com medicação local". Trab. Soc. Port. Dermat. Ven., **14** (1): 13, 1956.

135. Santos, I. A. dos: ,,O queijo da ,,Serra". Bol. Pecuário, **25** (1): 5—86, 1957.

136. Sobral, F. C. & Antunes, M. M.: ,,Tratamento Tópico das Tinhas". III Congr. Hisp. Port. Dermat., Santander, Set 1954; Act. Dermo Sifil., **8:** 1954.

137. Tanissa, A.: ,,O tratamento local das Tinhas tonsurantes". Gaz. Méd. Port., **1** (3): 589, 1948.

138. Tanissa, A.: ,,Action fongistatique et fongicide de l'huile de foie de mourue et d'un mélange d'acids gras fortement non saturés (thérapique, arachidonique et chepanodonique) provenant de cette huile". Ann. Dermat. Syph., Paris, **9** (6): 654, 1949.

139. Tanissa, A.: ,,Tinha tonsurante por infecção dupla (*T. granulosum* e *T. violaceum*). Gaz. Méd. Port. **3** (2): 385, 1950 e Ann Parasit., Paris, **24** (5—6): 547, 1949.

140. Tropa, E.: ,,Empoado" do bacalhau, considerações etiológicas". Ministério da Economia. Comissão Reguladora do Comércio do bacalhau. pgs. 1—18, 1956.

141. Tropa, E., Madeira, A., Bandeira, I., & Gavazzo, M. F.: ,,Estudos histopatológicos de orgãos de cavídeos e canídeos submetidos a administração de metabissulfito de potássio." An. Escola Méd. vet. Lisboa, **3:** 90—103, 1958.

142. Uden, N. van:,,Eine einfache Methode zum Studium der Pilzmorphologie im allgemeinen und der vegetativen Anastomosen im besonderen. Zugleich ein Beitrag zur Frage der systematischen Position des *Microsporum canis* Bodin (1902) und des *Microsporum langeroni* Vanbreuseghem (1950)". Arch. Derm. Syph., **193:** 468, 1951.

143. Uden, N. van: ,,Zur Kenntnis von *Torulopsis pintolopesii* sp. nov. Mit Beobachtungen über die parasitäire Phase von *Acladium castellanii* Pinoy." Arch. Mikrobiol., **17:** 199—208, 1952.

144. Uden, N. van, Almeida, R. C. & Singer, H.: ,,Histoplasmin sensitivity in Portugal" Port. Acta Biológica, **4:** 27—41, 1954.

145. Uden, N. van, Ré, L. & Ferreira, A. S.: ,,Preliminary study on histoplasmin sensitivity in Portuguese Africa, Goa and Macau" An. Inst. Med. Trop. **12:** 65—73, 1955.

146. Uden, N. van & Assis-Lopes, L.: ,,Sobre as leveduras do quefir". Bol. Pecuário, **38** (2): 3—27, 1955.

147. Uden, N. van, Matos-Faia, M. de & Assis-Lopes, L.: ,,The relationship between *C. claussenii* and *C. albicans*". Rev. Biologia **1** (1): 68—75, 1956.

148. Uden, N. van, Matos-Faria, M. de & Assis-Lopes, L.: ,,Isolation of *Candida albicans* from vegetable sources". J. gen. Microbiol. **15:** 151—153, 1956.

149. Uden, N. van & Carmo-Sousa, L. do: ,,Presumptive tests with liquid media for coliform organisms in yogurt in the presence of lactose fermenting yeasts". Dairy Indust., **22** (12): 1028—1029, 1956.

150. Uden, N. van & Assis-Lopes, L.: ,,Fermentation of raffinose in the absence of invertase by *Saccharomyces italicus* Castelli var. *melibiosi* nov. var." Port. Acta Biológica, A, **4** (4): 323—327, 1957.

151. Uden, N. van & Carmo-Sousa, L. do: ,,*Candida slooffii* nov. sp., a thermophilic and vitamin deficient yeast from the equine intestinal tract". Port. Acta Biológica, A **5** (1): 7—17, 1957.

152. Uden, N. van & Carmo-Sousa, L. do: ,,Yeast from the bovine caecum". J. gen. Microbiol. **16** (2): 385—395, 1957.

153. Uden, N. van & Carmo-Sousa, L. do: ,,Fungos do género *Aspergillus* no tracto intestinal de bovinos e equídeos". Bol. Pecuário, **25** (2): 31—49, 1957.

154. Uden, N. van, Carmo-Sousa, L. do & Farinha, M.: ,,On the intestinal yeast flora of horses, sheep, goats and swine". J. gen. Microbiol., **19** (3): 435—445, 1958.

155. Uden, N. van & Farinha, M.: ,,On the significance of temperature relations and vitamin deficiency patterns in the delimitation of yeast species''. Port. Acta Biológica, B, **4** (2): 161—178, 1958.

156. Uden, N. van, Braço-Forte Jr., M. C. & Carmo-Sousa, L. do: ,,The occurrence of *Cryptococcus neoformans* in the equine intestinal tract.'' Bull. Off. Int. Epizooties, **51** (1—2): 82—98, 1959.

157. Uden, N. van & Carmo-Sousa, L. do: ,,Further studies on the significance of temperature relations and vitamin deficiency patterns in yeast taxonomy''. Port Acta. Biológica, B, **4** (3): 239—256, 1959.

158. Villar, T.: ,,Considerações sobre dois novos casos de Aspergiloma pulmonar'', Gaz. Méd. Port., **12:** 117—133, 1959.

Papers not referred in the present review

Castellani, A.: ,,Some general remarks and experimental researches on symbiosis. The symbiotic gas-fermentation fenomenon (synergetic gas-fermentation phenomenon). Importance of symbiosis in the aetiology of certain diseases and certain symptoms of disease''. An Inst. Med. Trop. **10** (3) 2183—2207, 1953.

Castellani, A.: ,,Quelques considérations et expériences sur la symbiose. Le phénomène de fermentation gazeuse symbiotique. Maladies d'origine symbiotique''. Atti VI Congr. Int. Microbiol. **1**, 1, 319—330, 1953.

Castellani, A.: ,,Quelques considérations et expériences sur la symbiose''. Imp. Med. **17,** 251—263, 1953.

Castellani, A.: ,,A brief note on ,,bezoar''. Imp. Med. **18,** 5—7, 1954.

Castellani, A.: ,,Galactosaemia''. Brit. Med. J. **2,** 810, 1954.

Castellani, A.: ,,Some little-known tests. Mycological methods for the detection and identification of lactose, maltose, galactose and certain other carbohydrates copper-reducing and non-copper-reducing''. Imp. Med. **20:** 161—169, 1956.

Castellani, A.: ,,Mycological methods for the detection of galactosuria, maltosuria and lactosuria''. Indian J. Derm. **4** (2): 75—79, 1959.

Castellani, A.: ,,Mise en évidence mycologique du saccharose, glucide non réduteur''. Ann. Inst. Pasteur, **96,** 253—259, 1959.

Catanei, A.: ,,L'allergie et la prémunition mycosiques,'' Inst. Med. Trop. **6,** 307—323, 1949.

Catanei, A.: ,,Est-il facile d'étudier une mycose?'' An. Inst. Med. Trop. **6**, 273—286, 1949.

Catanei, A.: ,,Valeur des caractères cultureaux en mycologie médicale.'' An. Inst. Med. Trop., **6,** 289—303, 1949.

Leite, A. S. & Ré, L.: ,,Aspecto do *paracoccidioides brasiliensis* e do *Paracoccidioides cerebriformis* em saprofitismo''. An. Inst. Med. Trop. **8,** 27, 1951.

Nauck, E. G.: ,,Zur Histologie der Chromoblastomycose (Chromomycose)''. Gaz. Med. Port., **4:** 809—816, 1951.

Pujiula, P. J.: ,,Infection ovarica por un hongo microscopico''. Arq. Anat. Antropol. **24,** 631—636, 1946/47.

Uden, N. van: ,,Rapid method for testing raffinose fermentation by yeasts.'' J. Bacteriol. **72,** (6): 793—795, 1956.

LIBRI NOVI

Lewis, G. M., Hopper, M. E., Wilson, J. W. & Plunkett, O.A.: An Introduction to Medical Mycology. Fourth edition, 453 pp. Extensively illustrated. 1958. Year Book Publishers, Inc. 200 E. Illinois St., Chicago 11. Price $ 15. —

The first edition of this text appeared 18 years ago. When a text reaches its fourth edition in this relatively short span of time, there must be a basic reason for its popularity. The text alone how clear and concise it may be, does not account for this fact. There are quite a number of texts available in medical mycology in English, many quite superior to this text, which never have reached this popularity. It seems to be worthwhile to undertake a thorough analysis and to find out where the great shortcomings of the text lie and what are the roots and reasons for its unusual popularity.

The text covers in 41 chapters the whole field of human mycoses, superficial and systemic. In this edition the clinical and laboratory phases are not separated as it was the case in the previous editions. The senior author invited two new collaborators, J. W. Wilson and O.A. Plunkett. The former rewrote the chapter on deep mycoses, the latter contributed the chapter on contaminants.

The cooperation of mycologists with broad botanical background and of dermatologists with thorough mycological training is absolutely necessary in creating such a text as we are going to analyse. It is a true cooperation and not an overlapping endeavor. The mycologist with botanical background may know everything about taxonomy, physiology, biochemistry (nutrition) of dermatophytes. Yet he can never evaluate the etiologic-pathogenetic significance of fungi recovered upon or in human or animal tissues. The decision whether a fungus is etiological significant and the pathogenic agent in a given case remains the full responsability of the mycologically trained clinician. Thus, the staff of a mycological laboratory never can interpret microscopical and/or cultural findings as to their etiologic and/or pathogenic significance. The situation is similar to that in a serological laboratory. The serologist's reading of the result of a test is always correct. He reports what he had found. But the evaluation of his findings rest with the clinician alone.

The analysis of this text may consider several features: the part dealing with superficial mycoses, the discussion of deep-seated, systemic mycoses, the purely mycological contribution of a noted botanical scientist and the all important illustrative material.

To write any text which should carry the critical summary of existing knowledge at the given time to the uncomming youth, is a grave responsability. It presupposes the thorough knowledge of the field as far as humanly possible, the unbiased presentation of the material, the intellectual honesty and scientific integrity of the authors and the proper critical evaluation of the material to open up the flood gate for newer, better, more advanced research.

In the critical review of this text we may follow a reverse order to that mentioned above.

The chapter on Contaminants contributed by the noted mycologist, O. A. PLUNKETT is unqualifiedly excellent. The beautiful photographs of the giant cultures as well as those of the microcultures (slide cultures) and the clarifying schematic drawings are most instructive for the student of the material and contribute a great deal to broaden the knowledge of the wonder world of forms and structures of fungi. The accompanying text is clear, concise and sufficient.

WILSON's contribution on deep-seated (systemic) mycoses is the usual standard presentation of the subject. In this field there are no problems which would strain the diagnostic acumen of the author. Etiology and pathogenesis of systemic mycoses were settled long ago.

The substantial part of the presentation what is quite natural deals with the practically more important superficial mycoses. Here the text has most serious deficiencies for which the responsability may rest mainly with LEWIS. He reveals a detrimental inborn bias toward and an absolute scotom for new facts. The collaboration of WILSON did not alleviate but probably did aggravate the etiologic-diagnostic confusions and omissions in this area. HOPPER and PLUNKETT are not responsible for the failures and errors in this field, they rest exclusively with the clinicians, LEWIS and WILSON.

LEWIS and WILSON consistently and obstinately avoided to consider the newer etiologic and pathogenetic concepts which brought great clarification in the hodge-podge of superficial mycoses, concerning, in particular, "tinea pedis", "onychomycosis", "candidiasis". Their presentation gives a completely distorted idea to the inexperienced students concerning the field of superficial mycoses to the great detriment of the patients whom their former students will treat with a defective knowledge of dermatomycology, thirty years behind their time.

In the early years of medical mycology CAZENAVE dismissed the etiologic significance of fungi in human pathology as the "phantasy of microscopists". Eight decades later, fungi became so popular that everything turned into a "fungus disease" where fungi settled in the detritus masses of disorders of an entirely different etiology. The correction of this spirit will take a very long time. We find proof enough for that in medical history. Hundred years ago the "cause" of puerperal fever was universally taught by authorities of the first rank to be a "celestial-terrestrial-miasmatic" unknown. Just hundred years ago IGNATZ SEMMELWEISS published the result of his investigations demonstrating that the sole cause of puerperal fever was the *streptococcus*. Nonetheless, it took three more decades while mothers were dying all over the world until his live-saving discovery was "accepted". In biology, it is a sad fact, there is no forum like in the physical sciences, to call on the man to stand up to be counted.

In the etiologic and pathogenetic presentation of superficial mycoses LEWIS and WILSON failed as dermatologists. That holds true above all concerning "tinea pedis", manyfold source of all evil, among others the prime source of "id" eruptions. It is common knowledge that there are no superficial mycoses whose primary lesion is a deep-seated, sago-grain-like blister, embedded in the normal, not inflamed skin. They failed here also as mycologists. They assumed that any fungus found in and cultured from the detritus of a lesion has an etiologic and pathogenetic significance. They follow the line of those clinicians and mycologists who for decades cultured a host of the most different fungi from the scaly lesions of "pinta", until HERREJON came along and showed mycologists that pinta is a spirochaetosis.

Similarly they failed on both account concerning the diseases of the nails. They should have known that there are at present only three cases of true fungus infections of the nails, those published by STÜHMER years ago. From his findings we know that the nail plate and the nail matrix are never destroyed in true fungus infection of the nail. What LEWIS and WILSON call "onychomycosis" has nothing to do with fungi. The primary lesion in these instances is a deep-seated pustule, hematogen-endoparasitic in origine, which destroys the matrix up to complete cessation of the production of nail material. LEWIS and WILSON neatly assign a number of hyphomycetes and *Candida albicans* (!) to this process and they even know which type of fungus will cause which type of destruction! Among their therapeutic suggestions we find the "evulsion of the nail" as a "useful" method to clear up the nail conditions. They again omit their readers the better and greater experience of many others who observed that the nail after evulsion is growing back just as crookedly.

As "candidiasis" well established clinical and etiologic entities are presented which have nothing to do with *Candida albicans*.

Every chapter has its own fairly extensive bibliography. However, from foreign literature only the oldest ones are considered. Probably due to language difficulties the modern French, German and Italian literature is hardly considered. Its knowledge would have helped the authors to avoid serious mistakes and omissions.

One remark concerning prescription writing is in order. On page 239 there are three prescriptions which show the inconsistency of present day prescription writing. First of all, if the authors do not know Latin, they should not use it. The word petrolatum is neutral and the adjective to it is not "alba" but "album". Secondly, the authors mix languages, the prescription is half (bad) Latin and half English. Since our students do not know Latin and do not learn how a classical prescription should be written, the best is to write the prescriptions in plain English.

Between a pleasing hard cover zenith and nadir seldom met with such sharpness and consistency. But suffice now dealing with the nadir. The zenith is HOPPER'S work, the uncomparable illustrative material. The clinical cases are well selected, the photographs of the giant cultures as well as the most representative features in microcultures are superb. In black and white, nothing can improve on them. Mention should be made also of WILSON'S drawings which are outstanding in the concept of presentation of the details and have a great didactic value.

The Year Book Publishers deserve the highest praise for the most careful production of this text, the physical appearance of which is pleasing in every respect. The typography is excellent, the black and white illustrations and the color plates (there are two of them) are outstanding.

Due to the excellence of the illustrative material and its unique presentation this text will hold its popularity despite the most serious drawbacks in the text.

TIBOR BENEDEK

Plant Physiology, a Treatise. Vol. II. F. C. STEWARD, ed. Academic Press, Inc., New York, 1959, 758 pp.

The present volume is the first to appear (although numbered Vol. II) of a series of six volumes planned as a "synthesis of current knowledge" of the physiology of plants, written by authorities on each of the topics covered. While going far beyond the textbook exposition of the subject, both in critical depth and in references to experimental evidence and original literature, the series nevertheless will constitute a less voluminous survey, at a more moderate price, than the 18 volume "Handbuch der Pflanzenphysiologie" being published under the editorship of W. RUHLAND. The present volume consists of articles on permeability and transport through membranes by R. COLLANDER, water relations of cells by T. A. BENNET-CLARK, behavior of stomata by O. V. S. HEATH, absorption of inorganic salts by F. C. STEWARD & J. F. SUTCLIFFE, translocation of inorganic solutes by O. BIDDULPH, and transpiration and water economy of plants by P. J. KRAMER. These articles are admirably comprehensive and are made especially useful by the liberality with which experimental data have been included and the excellence with which halftone illustrations have been reproduced, for example BIDDULPH'S elegant radioautographs demonstrating redistribution of mineral elements within plants, on pp. 568—570. The 225-page article by STEWARD & SUTCLIFFE verges on the encyclopedic, covering and illustrating matters as diverse as the internal anatomy of higher plants, isolated cells in plant tissue cultures, and relations of intracellular and extracellular fluids in the animal body.

Naturally this volume has relevance largely to higher plants, some of the topics (e.g. stomata) pertaining only to them, while with others our knowledge of phenomena in lower plants — particularly fungi, for the readers of this journal — is rather fragmentary. However there are, for example, extensive treatments of permeability and active transport in the yeast cell and in bacteria, and information such as this can be readily found with the help of the carefully prepared organism and subject indices to the whole volume. In some areas, such as water relations, treatment of the behavior of lower organisms such as fungi and lichens might have made an interesting contrast, as well as providing a broader picture of plants in general. The intent in the remaining volumes does seem to be to cover important aspects of lower plant physiology, such as chemosynthesis in Vol. IB.

While no critical reader will, of course, find everything dealt with to his satisfaction , and I for example would like to have seen the basic concepts used in treating osmosis derived explicitly, and the rates of water movement in plants considered from a more quantitative point of view, yet all the articles seem to be extraordinarily rich in information while still unusually interesting to read. Some quaint editorial practices, such as the appearance, within the articles, of comments by the editor as footnotes, and the inclusion of introductory editorial "preambles" to the articles, can be noted. It is likely that these volumes will come to serve as valuable guides to the areas covered, and as invaluable sources of illustrative material in teaching.

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PETER M. RAY

VINCENT W. COCHRANE, *Physiology of Fungi*. John Wiley and Sons, Inc., New York, London. pp. xiii, 524, 79 text figures, 29 tables. 1958. $9.75.

The growing importance of biochemical processes based on the chemical activities of fungi is emphasized by the appearance of this third *Physiology of Fungi* in seven years, fourth book on fungus chemical activities in ten years. The book is well printed, with a minimum of typographical errors. Occasional digits in reference numbers may be transposed as the citation to HALL on page 358 which should read 124 instead of 214.

Each of the 14 chapters ends with a list of references, so that if each reference were cited only once 4,480 books, papers and reports would have formed the basis for this book. The chapter on nitrogen nutrition and metabolism lists 630 references, the introduction to carbon metabolism only 21.

Organisms considered in this treatment are the Fungi and Actinomycetes. The yeasts are generally omitted because "Professor Frank Dickens once spoke in a seminar to the effect that for the purposes of biochemistry the yeasts are to be considered animals." If the yeasts had been included, as well as the Myxomycetes and the Actinomycetes, in as great detail as those fungi which are considered, the book would have been much longer.

Subjects which are considered include cultivation and growth, composition of fungus cells, carbon nutrition and metabolism, respiration, nitrogen nutrition and metabolism, inorganic nutrition and metabolism, vitamin requirements, reproduction, spore germination, and the actions of physical and chemical agents. Some of the chapters or groups of chapters are ended with a concluding section summarizing the data presented and indicating points where additional work is required.

Throughout the book it is apparent that a critical need exists for more work to elucidate the concepts of mutants and heterocaryotic segregates. It seems to this reviewer that concepts based on the parasexual cycle in imperfect fungi need fuller explanation in the light of various kinds of biochemical work in recent years.

Chemical formulae, reaction sequences, metabolic pathways, and structures of compounds are given in considerable number and detail. Growth curves and tabular data are redrawn, compiled or summarized from original published data to conform with a style imposed by the author to obtain as much quantitative information as possible about the various types of processes considered.

Occasional statements may be questioned within a broader context than that in which they are made. The relationship between reproduction and spore germination and the dew point of the habitat of various strains of powdery mildews is ignored in discussions of water relations and of optimum temperatures for these phenomena. Laboratory experimentation tends to ignore the many facets of a natural environment so that the effect of vitamin or hormone-like substances produced by one fungus in a soil or other substrate on its neighbor may be overlooked or minimized..

On the whole, this book provides a ready reference volume for the student of biochemical physiology of fungi. While some physical aspects of fungal physiology are mentioned, this is usually in a chemical context. As the chemical aspects of this type of study become established, possibly more and more work can be carried out on physical aspects of nutrient transport, protoplasmic streaming, transpiration, cell wall structure, fruit body construction, spore discharge mechanisms, and many other similar problems which might complement and supplement the chemical processes toward the development of better knowledge of the fungi.

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Wm. Bridge Cooke

A Guide to the Identification of the Genera of Bacteria, 1st Edition, V.B.D. SKERMAN, University of Queensland, Brisbane, Australia: The Williams and Wilkins Company, Baltimore 2, Maryland 1959. 217 pp., $5,50.

The subject matter of this book is as follows: A Comprehensive Key to the Genera of Bacteria, Digest of Genera, Methods, A Guide to Study and the Index. The key is essentially the same as that in BERGEY'S Manual of Deterative Bacteriology, 7th edition, except that the author has included certain genera which were omitted from the Manual or appeared in the literature after the manuscript had gone to press. Also the material has been annotated more freely than in the previous key. Where certain information is controversial, care has been taken to present both sides of the issue.

The use of the Manual is often times considered awesome because of the necessary complexity of such a volume. The "Guide" obviates this problem considerably since by consulting this brief informative resume one can, in an orderly fashion, eliminate much of the laborious preliminary work of taxonomic studies. Then too, the author gives many valuable tips on coping with inherent problems. The reasonable price of the "Guide" will place a copy in the hands of many students and others who would not purchase the more complete but expensive Manual for their own library.

Preceding the comprehensive key to the Genera of Bacteria, the author explains the use of the key in an admirably simple manner and includes definitions of such things as a unicellular organism, a cell, a filament, a chain, a sheath, a capsule, etc. Under the Digest of Genera, the annotated "General Notes" contain useful gems of information culled from the author's rich experience and from other sources as well.

By way of a criticism, in the Digest of Genera covering *Actinomyces*, the author in discussing "Differentiating Characters" uses the term "free-living". Then later in the same description comments that the true habitat is unknown but that from sites of infection and history of cases all species probably occur in the soil or are normal inhabitants of body cavities. Several workers, whose papers have appeared over the last twenty years or more, have shown this organism to be a normal inhabitant of the mucous membranes of the mouth of man, swine and cattle. (E. EMMONS, T. ROSENBURY, J. SLACK, A. HOWELL and E. MEYER, to name only a few.) It has not been isolated from natural substrates as vegetable matter or other debris in the soil and those of us who have worked with this fastidious organism doubt that it ever will be. Probably the author, who has graciously elicited criticism from readers, will consider these suggestions in later editions.

In "Methods" the author points out that, in the interest of a more uniform approach to investigative work he has selected methods from many sources most of which have been tested in his own laboratory. Although these methods are not to be regarded as standard, everyone agrees a clearer picture can be obtained when there is uniformity in the materials and methods employed in various laboratories. One cannot over-estimate the value of this section for it will save considerable time and effort for many. Preceding the index, there is a "Guide to Study" which is a brief, helpful compilation of selected groupings of bacterial genera.

In the opinion of the reviewer "A Guide to the Identification of the Genera of Bacteria" makes a significant contribution to the field of bacterial taxonomy.

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Gallery of Contemporary Noted Mycologists

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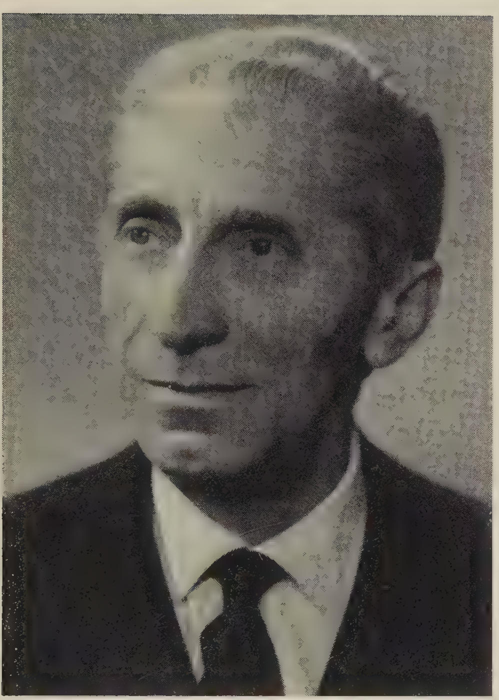

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Gallery of Contemporary Noted Mycologists

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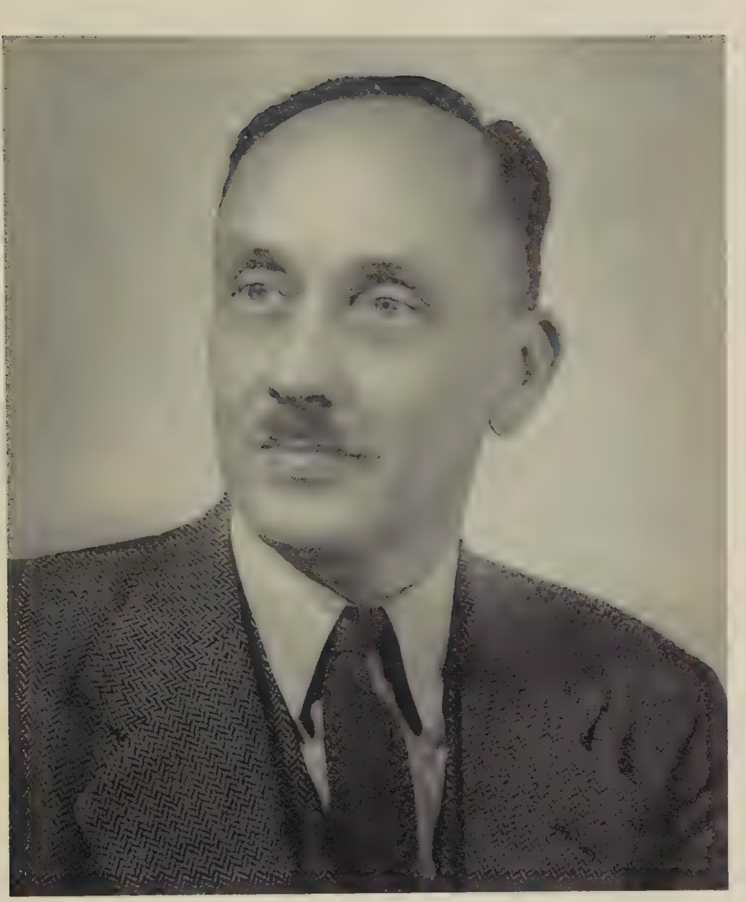

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COMPARATIVE PHYSIOLOGY OF SOME REPRESENTATIVES OF THE GYMNOASCACEAE [1])

by

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(18.III.1960)

The family Gymnoascaceae comprises a group of primitive Ascomycetes of the order Aspergillales. The Gymnoascaceae are world wide in distribution although they have been reported most frequently from warmer climates. These fungi occur most frequently as saprophytes on a variety of substrates, including feathers, fur, nails, hooves, bones, dung, cadavers, decaying plant material, paper and soil. Certain Gymnoascaceae have been isolated from pathogenic conditions of skin and hair of man and animals. Because of this supposed pathogenicity in addition to certain similar cultural and morphological features the dermatophytes or Trichophytoneae (DODGE, 1935) have been presumed to represent the imperfect stages of certain Gymnoascaceae. Reports of formation of asci by a few species of the Trichophytoneae have tended to support this presumption. However, later workers have been unable to confirm these reports. The relation of the Trichophytoneae to the Gymnoascaceae is still controversial. Since there are excellent reviews covering these reports, the reader is referred to them for pertinent literature (see TATE, 1929, EMMONS, 1931, GREGORY, 1935).

Studies of the Gymnoascaceae have been confined for the most part either to morphological studies or to combined morphological and cytological studies. This work has been summarized or reviewed by others (BENJAMIN, 1956, KUEHN, 1955, et seq.). KUEHN (1958, 1959) presented a taxonomic treatment of the entire family.

Very little has been published concerning the physiology of species of Gymnoascaceae, whereas numerous studies on the physiology of related groups, such as the Trichophytoneae and the Aspergillaceae, have been reported. VOLKONOSKY (1934) apparently was the first to present results on nutritional studies of the Gymnoascaceae. He compared the nutrition of *Ctenomyces serratus*, a saprophyte, with that of certain dermatophytes, including *Sabouraudites felineus* OTA and LANGERON (= *Ectotrichophyton felineum* (BLANCHARD) CASTELLANI and CHALMERS), *Epidermophyton floccosum* (HARZ) LANGERON and MILOCHEVITCH, *Trichophyton crateriforme* SAB. (= *T. tonsurans* MALMSTEN), *T. rubrum* SEMON, *Ctenomyces granulosum* SAB. and *Ctenomyces asteroides* SAB. He employed alanine and potassium nitrate as the nitrogen sources. The results showed that all of the fungi tested readily utilized alanine but none could utilize nitrate.

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[1] This paper represents a portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany in the University of Illinois.

[2] The writer wishes to express her sincere appreciation to Professor LELAND SHANOR, under whose direction this investigation was carried out, for his advice and encouragement. The author is endebted to Dr. HAROLD H. KUEHN for reading the manuscript and for his many helpful suggestions in its revision.

VOLKONOSKY also found that the utilization of carbohydrates by these fungi was extremely slow. He observed that *C. serratus* did not ferment sorbite. The reaction of the medium invariably became slightly alkaline with his test fungi.

HAWKER (1936) observed that *Gymnoascus setosus* grew and fruited well on a mineral-dextrose medium which contained potassium nitrate. The addition of lentil extract to this medium did not stimulate growth.

According to SCHOPFER & BLUMER (1940) *G. setosus* grew on a mineral-dextrose medium with aspargine as the nitrogen source. The addition of thiamin or its intermediates did not affect growth in any manner.

According to LATHAM (1909), BERTHELOT in 1893 claimed that a species of *Gymnoascus* fixed nitrogen. This has not been confirmed.

ROSENBAUM (1944) reported on the cultural requirements of *Arachniotus trisporus*, shown to represent *Byssochlamys nivea* WESTLING by BENJAMIN (1956) and KUEHN (1957). She showed that a liquid medium with 1% peptone and 2% glucose or sucrose permitted the best growth. Lactose also enabled the fungus to grow. ROSENBAUM was the first investigator to report studies on pH and temperature relations of any of the Gymnoascaceae. Good growth of *B. nivea* was obtained at pH 6.4 and 7.0. Usually the fungus lowered the pH to 6.0 or below. The optium temperature for growth of *B. nivea* was found to be 30—35° C, and most probably about 33° C.

In another series of experiments ROSENBAUM determined the effect of various concentrations of carbohydrate and peptone upon the growth and production of various morphological structures of this fungus. She concluded that different levels of glucose and peptone had little or no effect upon the size, form or time of production of the morphological features of *B. nivea*. ROSENBAUM grew this fungus on agar which contained only glucose or peptone and found that both were required for normal growth.

KUEHN & CROSBY (1959), working with *Pseudoarachniotus roseus*, performed an extensive nutritional study. They investigated carbon, nitrogen, vitamin and other growth requirements in shaken flask cultures. The optimum conditions for *P. roseus* proved to be the following: maltose, dextrin, xylose or soluble starch as the carbon source; ammonium acetate or asparagine as the nitrogen source; initial pH 7.0—8.0; 37° C; KH_2PO_4 and $MgSO_4.7H_2O$. Thiamin and biotin were found to stimulate growth, while additions of various trace metals did not.

The purpose of the present investigation was to determine the nutritional requirements of selected representatives of different genera of the Gymnoascaceae and to ascertain how closely the results obtained were similar to those reported from studies of the Trichophytoneae and the Aspergillaceae.

Materials and Methods

The fungi used in this investigation were as follows: *Myxotrichum uncinatum* (Eidam) Schroeter, isolated by R. K. Benjamin from goat dung, March, 1949, Urbana, Illinois; *Ctenomyces serratus* Eidam, obtained from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands; *Gymnoascus reessii* Baranetzky, isolated by R. K. Benjamin from goat dung, September, 1948, Urbana, Illinois; *Eidamella deflexa* (Berk.) Benjamin, a subculture of DeLamater's isolate obtained from the Centraalbureau voor Schimmelcultures; *Arachniotus reticulatus* Kuehn, isolated by C. W. Emmons from bat dung, Georgia; and *Penicillium spiculisporum* Lehman, isolated by the author from soil, April, 1953, Urbana, Illinois. *Penicillium spiculisporum*, a representative of the Aspergillaceae, was included in this investigation because the nature of its ascocarp is reminiscent of a gymnoascaceous structure. A species closely related to this fungus, *P. luteus* Zukal, was classified in the Gymnoascaceae by Saccardo (1895), although other investigators did not agree (Raper & Thom, 1949). It seemed desirable, therefore, to compare the nutrition of *P. spiculisporum* with that of typical gymnoascaceous species.

Preliminary tests were carried out to determine a satisfactory growth medium. The following media were used: (1) glucose-asparagine medium (Lilly & Barnett, 1951); (2) Steinberg's dibasic nutrient medium (Steinberg, 1941); (3) Czapek's solution (Czapek, 1902, Dox, 1910); (4) Robbins and Ma's basal medium (Robbins & Ma, 1945). The glucose-aspargine medium appeared to be the most satisfactory and it has been utilized throughout the present investigation. This medium has the following composition:

10.0 g	glucose
2.0 g	asparagine
1.0 g	KH_2PO_4
0.5 g	$MgSO_4.7H_2O$
100.0 μg	thiamin HCl
5.0 μg	biotin
2.0 ml	microelement stock soln.
	doubly-distilled water to make one liter

The microelement solution was prepared by dissolving the following salts in 600 ml doubly-distilled water: 723.5 mg $Fe(NO_3)_3.9H_2O$, 439.8 mg $ZnSO_4.7H_2O$ and 203.0 mg $MnSO_4.4H_2O$. The basal medium was prepared in lots of 4—5 liters which were double the final strength. After the microelements were dissolved in water, concentrated sulfuric acid was added to make a clear solution and doubly-distilled water was used to bring the volume up to one liter. Each ml of this solution contained 0.1 mg each of iron and zinc and 0.05 mg of manganese. The microelement stock solution was stored in the refrigerator.

In the nitrogen assimilation studies the following nitrogen sources were tested: sodium nitrate, ammonium sulfate, ammonium nitrate, asparagine, glycine, lysine HCl, methionine, leucine, tyrosine, proline and glumatic acid. The basal medium was prepared without a nitrogen source. Two gram amounts of the nitrogen source to be tested were added to 500 ml of the basal medium. The pH was adjusted to 6.5 with 6 N NaOH after which the volume was brought up to 960 ml with doubly-distilled water. Twenty-four ml aliquots of this medium were distributed into 125 ml Erlenmeyer flasks which were then autoclaved for 15 min. at 15 lbs. pressure. Following this one ml of a sterile thiamin solution was added aseptically to the flasks to bring the volume of each flask up to 25 ml.

For the carbon utilization studies the same general procedure was employed except that the carbon source was omitted from the basal medium. The carbohydrates to be tested were added to the medium in the same manner as outlined for the nitrogen studies. The carbon sources which were tested included: glucose, fructose, galactose, maltose, sucrose, lactose, raffinose, xylose, dextrin and sodium lactate.

The same general procedure was followed in the vitamin requirement tests with certain variations. In the vitamin study the basal medium was prepared in double strength without the addition of the vitamins. Vitamin-free casein hydrolysate was used as the nitrogen source instead of asparagine and the medium was boiled with activated charcoal (Norit, 5 g/l) in order to render it essentially vitamin-free (LILLY & BARNETT, 1951). The charcoal was removed by filtration and the microelements from the stock solution were added. The medium was ditributed in 500 ml aliquots to flasks each of which contained a measured quantity of a certain vitamin solution to be tested. If thiamin was to be added later the volume of the basal medium in each flask was made up to 960 ml and the same subsequent procedure was followed as in the nitrogen and carbon studies. However, if thiamin was not to be included in the medium, the volume of the basal medium in each flask was brought to one liter. The basal medium then was distributed to 125 ml Erlenmeyer flasks in 25 ml amounts and the flasks were autoclaved. The cotton plugs were covered with cheese cloth to prevent cotton fibers from serving as a source of vitamin contamination. The vitamins which were tested included: thiamin, 100 μg/1; pyridoxine HC1, 100 μg/1; biotin, 5 μg/1; calcium pantothenate, 100 μg/1 and inositol, 5 mg/1.

Experiments were performed to elucidate the trace element requirements of the test fungi. The same general procedure was followed as before with certain variations. In the preparation of the basal medium the trace element sources were omitted. The medium was treated with $CaCO_3$ to remove trace element contamination (STEINBERG, 1919). Asparagine was not included in the basal medium since amino acids form non-ionized complexes with micro-element impurities and the latter can not be removed by $CaCO_3$ treatment. Instead, a suitable mixture of asparagine and thiamin was prepared and this mixture was treated with dithizone (STOUT & ARNON, 1939) in order to remove heavy metal contamination. Then the mixture was passed through an ultra-fine, fritted-glass filter and was added aseptically to the culture flasks.

After treatment with $CaCO_3$ the basal medium was adjusted to pH 6.0, rather than to 6.5 as in the other experiments, in order to prevent possible precipitation of salts in the alkaline medium. The basal medium was distributed in 12 ml aliquots to the culture flasks. Different combinations of the trace element solutions were added to the test flasks. The quantity of these trace element solutions varied according to the combinations of elements used. Finally, the medium in each flask was diluted with doubly-distilled water to give a final volume of 24 ml.

Dibasic potassium phosphate was substituted in the basal medium for the monobasic variety since the latter can not be treated with $CaCO_3$ (STOUT & ARNON, 1939). Four g/1 of $Na_2HPO_4.12H_2O$ were added to the basal medium to supplement the phosphate which might have been removed by the purification process. A control flask was included which had all four trace elements minus the $Na_2HPO_4.12H_2O$. The compounds employed as sources of trace elements, used singly and in combination, included: $Fe(NO)_3.9H_2O$, 0.2 mg/1; $ZnSO_4.7H_2O$, 0.2 mg/1; $MnSO_4.H_2O$, 0.1 mg/1; $CuSO_4.5H_2O$, 0.075 mg/1.

The cotton plugs were covered with cheese cloth to prevent cotton fibers from possibly contaminating the medium with trace elements.

Inoculum was grown for five days on a reciprocal shaker at 26° C in 125 ml Erlenmeyer flasks with 25 ml Sabouraud's peptone-dextrose broth. Under such conditons the fungi formed a heavy growth of approximately uniform small pellets of mycelium. A 0.5 cm diam. platium loopful of such growth was used to inovulate all experimental flasks. In the vitamin and trace element experiments the medium was removed from the inoculum by centrifuging it and washing several times with re-distilled water. In the trace element studies after a given flask was inoculated and prior to inoculation of the next flask the platinum loop was dipped into conc. HC1, following which the loop was heated in a flame. This process was repeated until no colored flames were visible. This was done to avoid the transfer of different heavy metals from one test flask to another.

Preliminary tests were carried out to determine the incubation period necessary for optimum growth and to establish the optimum pH. In general, pH 6.0-6.5 was found to be the optimum range, while an incubation period of 12 days produced the maximum dry weight after which there was a weight loss due to autolysis.

Cultures were incubated for 12 days on a reciprocating shaker at 26° C. The flasks were then autoclaved briefly to kill the fungi and the growth was separated from the medium by filtration using tared filter papers. The filter papers were dried for 3 days at 95° C and re-weighed to establish the dry mycelial weight. Experiments were run in triplicate and all figures represent the average values. The initial pH was recorded after autoclaving and the terminal pH was recorded after harvesting the mycelium. All glassware was Pyrex and was cleaned with cleaning solution, washed with tap water, then with distilled water and finally with re-distilled water.

RESULTS AND DISCUSSION

Since the Gymnoascaceae are generally considered to be related to the Trichophytoneae as well as to the Aspergillaceae the result of our study will be discussed comparatively with reported findings concerning these other groups of fungi.

I. Nitrogen assimilation.

A. Gymnoascaceae.

Table I presents the results of the nitrogen assimilation studies. *Myxotrichum uncinatum* grew fairly well with $NaNO_3$ as the sole nitrogen source but did not utilize ammonium nitrogen. All of the organic compounds tested supported good growth of *M. uncinatum*, although the amino-acid mixture was the best source of nitrogen.

Eidamella deflexa, although it grew very slowly with a smaller final cell yield, was similar to *M. uncinatum* in its nitrogen requirements. The only inorganic nitrogen source utilized by *E. deflexa* was $NaNO_3$. All the organic sources supported growth, however, asparagine permitted maximum growth.

Heretofore, *Gymnoascus setosus* had been the only species of the genus on which nutritional studies had been performed (HAWKER, 1936, SCHOPFER & BLUMER, 1940). In the present investigation it was found that *G. reessii* grew poorly on all inorganic nitrogen sources. The aminoacid mixture supported excellent growth but casein hydrolysate permitted only fair growth.

VOLKONOSKY (1934) reported that *Ctenomyces serratus* did not utilize inorganic nitrogen but could use alanine. Our results support his observations. Only the amino acid mixture and casein hydrolysate supported growth of this fungus.

TABLE I

NITROGEN ASSIMILATION BY FUNGI TESTED

	CONTROL 1 No nitrogen	$NaNO_3$	NH_4NO_3	$(NH_4)_2SO_4$	ASPARAGINE	GLYCINE	Mixture of AMINO ACIDS	Casein hydrolysate
Initial pH	5.5	6.1	6.1	6.3	6.4	6.3	6.3	6.3
MYXOTRICHUM UNCINATUM	▲6.6 ↑ 6.6	8.2 53.3	3.5 4.7	3.4 7.8	7.0 79.2	7.5 71.6	7.0 95.4	6.5 73.4
EIDAMELLA DEFLEXA	6.0 0	7.6 16.5	3.7 0	3.7 0	6.7 45.0	4.3 22.8	5.6 12.5	6.0 22.0
GYMNOASCUS REESSII	6.4 6.5	6.2 7.3	5.8 12.2	5.5 15.5	7.1 11.2	6.2 20.6	6.3 103.1	5.4 36.7
CTENOMYCES SERRATUS	7.0 11.5	6.3 6.0	5.0 8.8	4.0 8.8	7.1 15.9	7.1 17.2	7.1 91.6	6.0 65.3
ARACHNIOTUS RETICULATUS	6.3 4.6	6.2 2.6	5.1 2.4	5.1 4.6	7.0 22.2	7.3 45.1	6.1 68.3	5.0 19.3
PENICILLIUM SPICULISPORUM	3.6 12.1	6.6 55.0	6.4 45.9	3.5 39.0	6.9 73.1	6.9 63.7	7.0 52.8	6.1 21.2

In each block, the top figure represents the final pH, the middle figure indicates the mg of dry weight, and the height of the columns indicates the relative amount of growth.

Arachniotus reticulatus could not utilize inorganic nitrogen. However, it grew well on all the organic sources, of which the amino acid mixture supported the best growth.

Asparagine was the best nitrogen source for *Penicillium spiculisporum*. However, this species utilized both inorganic and organic nitrogen.

In controls which lacked a nitrogen source all species, except *E. deflexa*, which did not grow at all, exhibited only slight growth ranging from 6.5 to 12.1 mg. Since growth of *P. spiculisporum* and *C. serratus* seemed to be significantly great the possibility exists that these fungi are able to utilize atmospheric nitrogen. However, further specific tests must be accomplished prior to arriving at a definite conclusion. Previous reports of nitrogen fixation include those of BERTHELOT in 1893 for *Aspergillus niger* and a *Gymnoascus* sp. (LATHAM, 1909), SIDA (1901) and LATHAM (1909) for *A. niger* and PURIEWITSCH (1895) for *Penicillium glaucum*. Nitrogen fixation has been denied by ALLISON, et al. (1934) for *A. fuscus*, *A. cinnamomeus*, *A. niger* var. *altipes* and 8 strains of *A. niger*, ROBERG (1932) for 14 species of *Aspergillus*, SCHROEDER (1931) for 13 strains of *A. niger* and DUGGAR & DAVIS (1916) for *A. niger*.

B. *Trichophytoneae.*

VOLKONOSKY (1934) reported that *Trichophyton crateriforme*, *T. rubrum* and *Epidermophyton floccosum* did not utilize inorganic nitrogen but did assimilate alanine. MOSHER, et al. (1936) reported that *T. mentagrophytes* did not grow on a synthetic medium without additional amino acids. ROBBINS & MCVEIGH (1946) showed that *T. mentagrophytes* was inhibited by 60 ppm hydroxyproline. A similar response was obtained with *T. gypseum*, T. *purpureum*, *E. floccosum* and *Microsporon canis*, but species of *Aspergillus* and *Penicillium* were not inhibited.

C. *Aspergillaceae.*

STEINBERG (1939a) investigated the utilization of inorganic nitrogen by *Aspergillus niger* and concluded that the best sources for growth were ammonium salts, and nitrated and nitrohydroxylaminic acid salts. TAMIYA & USAMI (1940) reported that *A. oryzae* exhibited good growth in a synthetic medium which contained inorganic nitrogen. SAKAMURA (1930, 1934), who worked with *A. oryzae*, attributed the relative assimilability of ammonium and nitrate nitrogen to miscellaneous environmental factors such as acidity of the medium and influence of iron and copper. The first view was favored by ITZEROTT (1936) who found that trace elements were ineffective in influencing assimilation by *A. niger*. WOOSTER & CHELDELIN (1945) reported that asparagine was a good source of nitrogen for *Penicillium digitatum*.

According to STEINBERG (1950), quantitative studies of amino acid utilization must be interpreted on the basis of the type of nitrogen assimilation which the fungus possesses. A fungus that is unable to use inorganic nitrogen may be assumed to use amino acids. Other fungi which can assimilate inorganic nitrogen may be assumed to use amino acids. Other fungi which can assimilate inorganic may make use of ammonium nitrogen only subsequent to deaminizations. *Aspergillus niger* falls into the latter group.

A quantitative study (STEINBERG, 1942a) of the growth of *A. niger* with amino acids disclosed that these compounds could be arranged into three groups. The nitrogen in alanine, arginine, aspartic acid, glutamic acid, glycine, hydroxyproline, ornithine and proline proved to be equivalent to inorganic nitrogen. The use of serine, threonine and tryptophane resulted in yields approximating 50% nitrogen assimilation. The other amino acids permitted very poor growth. STEINBERG termed the first group the primary and the second group the secondary amino acids.

D. *Summary of nitrogen assimilation.*

ROBBINS (1937) classified the fungi into 4 groups according to their ability to utilize various nitrogen sources. His classification is as follows:

	N_2	NO_3	NH_3	Organic
Group I	+	+	+	+
Group II	—	+	+	+
Group III	—	—	+	+
Group IV	—	—	—	+

Fungi other than those which ROBBINS included in his work also fit into his classification (STEINBERG, 1939b). Thus, *A. fisheri* (WENCK, PETERSON & FRED, 1935), *A. oryzae*, (YAMAGATA, 1934), and *P. chrysogenum* (MOYER, MAY & HERRICK, 1936) are included in group II. However, exceptions have been reported among other fungi (STEINBERG, 1950, LILLY & BARNETT, 1951).

We have classified the fungi studied in the present investigation, and certain other fungi discussed above, into ROBBINS system as follows:

Group II. *A. niger*, *A. oryzae*, *A. fisheri*, *P. chrysogenum*, *P. spiculisporum* and *P. javanicum*.

Group IV. *Ctenomyces serratus*, *C. asteroides*, *C. granulosum*, *Gymnoascus reessii*, *Arachniotus reticulatus*, *Trichophyton crateriforme*, *T. rubrum*, *Ectotrichophyton felineum* and *Epidermophyton floccosum*. Our studies have also revealed that *Myxotrichum uncinatum* and *Eidamella deflexa* utilize nitrate and organic nitrogen but do not utilize ammonium nitrogen and thus fall into none of the categories of ROBBINS. The reason for this is probably that the medium became too acidic (pH 3.4—3.7) for growth of these fungi. This phenomenon is frequently encountered when ammonium nitrogen is rapidly assimilated.

II. Carbon assimilation.

A. *Gymnoascaceae.*

The results which have been obtained in our studies are recorded in Table II. No growth or only negligible growth was observed in the control medium which lacked a carbon source.

Myxotrichum uncinatum assimilated most of the carbohydrates tested. Raffinose, sucrose and dextrin supported excellent growth and maltose, glucose, fructose, galactose and xylose were good sources of carbon. Utilization of lactose was slow resulting in poor growth after 12 days but good growth after 28 days incubation. *Myxotrichum uncinatum* could assimilate sodium lactate although growth was poor on this source of carbon. In general, this fungus utilized the complex carbohydrates more readily than the hexoses.

Eidamella deflexa was similar to *M. uncinatum* in its ability to utilize all the sources of carbon. However, the rate of growth of *E. deflexa* was slower than that of *M. uncinatum*. Xylose and raffinose supported the best growth of *E. deflexa*. Lactose was more available and sodium lactate was less available than with *M. uncinatum*. DODGE (1935) placed *Gymnoascus setosus* in synonymy with *E. spinosa*, now *E. deflexa*, although DELAMATER (1937) disclaimed this synonymy. The results obtained by HAWKER (1936) for *G. setosus* seem to agree with the results reported here for *E. deflexa*. Thus, results of nutritional studies tend to support the synonymy of these two species.

Gymnoascus reessii either did not utilize any of the carbohydrates or utilized them very slowly. When some of the flasks were incubated for 28 days reasonably good growth resulted.

Ctenomyces serratus utilized only fructose. VOLKONOSKY (1934) reported that *C. serratus* assimilated glucose and fructose but not galactose, maltose, sucrose, lactose, raffinose or dextrin. Our result support his observation except that growth obtained was very poor on glucose.

Maltose, fructose and glucose were utilized by *Arachniotus reticulatus*, although maltose supported the best growth.

Penicillium spiculisporum assimilated all the carbon sources with equal efficiency except lactose and sodium lactate, while raffinose was only poorly assimilated.

The terminal pH of the culture filtrates, in most cases, showed an alkaline tendency. However, the filtrates of *Eidamella deflexa* and *Arachniotus reticulatus* with maltose as the carbon source and of *E. deflexa* with xylose were acidic.

B. *Trichophytoneae.*

BODIN (1899, 1902), GODDARD (1934) and MOSHER, et al. (1936) reported that *Trichophyton mentagrophytes* (*T. interdigitale*) grew well on glucose, fructose, galactose and maltose but grew poorly or not at all on sucrose, lactose, and lactate. These investigators stated

TABLE II

Carbon assimilation by the fungi tested.

	CONTROL No carbon	DEXTROSE	FRUCTOSE	GALACTOSE	MALTOSE	SUCROSE	LACTOSE	RAFFINOSE	XYLOSE	DEXTRIN	Na-LACTATE
Initial pH	6.3	6.1	5.9	6.1	6.3	6.2	6.4	6.3	6.0	6.3	6.3
MYXOTRICHUM' UNCINATUM	7.6 ▲ * 7.1 ↑	6.8 87.1	6.8 83.7	6.9 81.0	6.9 84.1	6.8 96.5	7.2 * 17.4	6.6 99.0	7.0 76.0	7.1 92.1	9.6 42.3
EIDAMELLA DEFLEXA	6.8 0	6.0 42.8	6.5 24.7	6.9 41.1	5.8 38.4	7.1 39.4	7.0 44.5	6.6 46.9	5.8 49.2	7.0 33.3	6.8 9.0
GYMNOASCUS REESSII	7.1 6.4	6.9 12.0	6.5 8.1	6.9 10.5	7.1 * 9.5	7.1 * 14.7	7.0 6.3	7.2 * 6.4	6.6 7.4	7.3 * 10.7	7.2 5.6
CTENOMYCES SERRATUS	7.0 0	7.1 17.6	6.0 88.3	7.0 13.5	6.9 6.9	6.9 5.9	6.9 6.5	6.9 0	6.6 6.8	7.1 15.1	7.2 5.0
ARACHNIOTUS RETICULATUS	7.4 8.6 *	6.6 29.9	6.6 51.4	6.8 11.1	5.5 73.0	7.0 12.3 *	7.4 9.2	7.6 7.2	6.8 8.9	7.3 10.0 *	7.4 5.9
PENICILLIUM SPICULISPORUM	6.6 0	6.4 66.5	6.5 64.4	6.7 74.8	6.5 66.0	6.7 73.4	6.7 5.1	6.6 27.1	6.7 73.2	6.6 59.1	7.2 6.6

In each block the top figure represents the final pH, the middle figure indicates the mg of dry weight, and the height of the column indicates the relative amount of growth. The * indicates the formation of crystals in the culture filtrates.

that in a synthetic medium with minerals, amino acids and vitamins this fungus grew to maturity in the absence of a carbohydrate. *Microsporon canis* was similar to *T. mentagrophytes* except that the former grew on dextrin but could not assimilate galactose or lactose (BODIN, 1899; GODDARD, 1934). These results agreed with those obtained by TATE (1929) in a study of enzymes of Trichophytoneae, except that he did not find sucrose to be present.

VERUJSKY (1887) reported that *T. schoenleinii* did not utilize sugar from any source, whereas *T. tonsurans* assimilated glucose but not sucrose. VOLKONOSKY (1934) found that *T. rubrum* and *Epidermophyton floccosum* could not utilize certain carbon sources and that *T. crateriforme* utilized glucose but not fructose.

C. *Aspergillaceae.*

The assimilation of carbon by *Aspergillus oryzae* (TAMIYA, 1932) is very similar to that of *Myxotrichum uncinatum*. TAMIYA stated that growth of *A. oryzae* was often better on polysaccharides than on hexoses. This species grew well on dextrin, raffinose, sucrose, maltose and fructose, good on galactose, glucose and xylose, poorly on lactate and not at all on lactose.

LOCKWOOD (1933) found that *Penicillium* grew poorly on sucrose. STEINBERG (1939a, 1942b) showed that glucose, fructose, mannose, sorbose, and xylose were good sources of carbon for *A. niger*, while galactose, lactose, glycerol and mannitol were poor sources.

D. *Summary of carbon utilization.*

The following groupings of fungi can be made on the basis of our findings and those reported in the literature.

Group 1. Fungi able to utilize most of the carbon sources tested are *Aspergillus oryzae, A. fischeri, Myxotrichum uncinatum* and *Eidamella deflexa.*

Group 2. Fungi able to utilize fewer carbon sources than the fungi of Group 1 include *Aspergillus niger, Penicillium spiculisporum, P. chrysogenum, P. javanicum, Microsporon canis* and *Trichophyton mentagrophytes.*

Group 3. Species capable of assimilating glucose and/or fructose include *Ctenomyces serratus, Arachniotus reticulatus, Trichophyton crateriforme* and *T. tonsurans.*

Group 4. Fungi unable to rapidly utilize any carbon source are *Gymnoascus reessii, Trichophyton rubrum, T. schoenleinii* and *Epidermophyton floccosum.*

III. Vitamin requirements.

It is evident from the results of the vitamin studies shown in Table III that all the fungi produced good growth in the control medium lacking vitamins. Addition of vitamins in different combinations had some, little, or no effect, depending upon the species involved.

TABLE III

Vitamin requirements of the fungi tested.

	CONTROL I Untreated complete medium	CONTROL II No vitamin added	CONTROL III Th+ Bi+ In+ Py +Pa added	Thiamin omitted	Biotin omitted	Inositol omitted	Pyridoxin omitted	Pantothenate omitted
Initial pH	6.3	5.5	5.5	5.6	5.6	5.6	5.6	5.5
MYXOTRICHUM UNCINATUM	▲ 6.5 ↑ 73.1	6.4 61.4	6.4 61.3	6.4 65.4	6.7 58.8	6.3 57.7	6.3 54.0	6.4 61.5
EIDAMELLA DEFLEXA	6.0 22.0	4.7 28.9	5.8 31.4	4.8 26.1	6.1 31.4	4.3 28.4	5.5 31.0	5.0 29.2
GYMNOASCUS REESSII	5.6 36.7	6.1 20.4	6.0 25.2	6.2 42.0	6.5 24.4	6.1 28.6	6.1 26.0	6.2 32.7
CTENOMYCES SERRATUS	6.0 65.3	6.3 60.6	6.3 71.5	6.3 56.5	6.1 70.3	6.3 66.3	6.1 72.9	6.2 74,4
ARACHNIOTUS RETICULATUS	19.3	29.3	21.4	Th + ↓ 44.1 23.8	Bi + ↓ 33.3 43.4	In + ↓ 27.1 47.9	Pyrd + ↓ 51.6 40.2	Pant + ↓ 28.6 48.8
PENICILLIUM SPICULISPORUM	6.1 21.4	5.8 42.4	5.8 47.0	6.0 55.8	6.0 47.3	5.8 45.1	6.1 38.2	5.8 39.6

In each block, the top figure represents the final pH, the middle figure indicates the mg of dry weight, and the height of the column indicates the relative amount of growth.

Ctenomyces serratus was stimulated by thiamin. *Arachniotus reticulatus* showed a partial deficiency for thiamin and pyridoxine. However, the control containing all the vitamins supported less growth than the control lacking all the vitamins. Presumably the presence of some vitamins for which the fungus was self-sufficient caused this growth depressing effect (LILLY & BARNETT, 1951). Therefore, a trial experiment was accomplished in which different vitamins were added singly to the vitamin-free basal medium. A greater increase in growth resulted in media containing thiamin and pyridoxine singly than in media lacking these vitamins. Pantothenate, inositol and biotin were found to depress growth when they were present singly. Similarly, thiamin had a depressing effect on growth of *Penicillium spiculisporum* since growth increased when thiamin was lacking. The same mechanism could explain less growth on untreated media which probably contained thiamin. *Penicillium spiculisporum* also showed a partial deficiency for pyridoxine and pantothenate.

Gymnoascus reessii exhibited a partial deficiency for biotin and pyridoxine. *Myxotrichum uncinatum* and *Eidamella deflexa* were not influenced by the addition of vitamins in combinations. It appears that these species are completely autotrophic for vitamins.

The following classification can be achieved based on our results and those reported in the literature:

Group I. Fungi autotrophic for vitamins include *Myxotrichum uncinatum*, *Eidamella deflexa* and most species of *Aspergillus* and *Penicillium* (LILLY & BARNETT, 1951, and ROBBINS & KAVANAGH, 1942).

Group II. Fungi partially deficient for vitamins include (the stimulating material is indicated in parenthesis) *Penicillium notatum* (thiamin and yeast extract), *P. digitatum* (orange juice), *P. glaucum* (yeast extract), *Aspergillus niger* (yeast extract and bios), *Microsporon fulvum* (amino acids and yeast extract), *Trichophyton crateriforme* (rice polishing), *T. discoides* (thiamin), *T. roseum* (thiamin and rice polishing) (all according to ROBBINS & KAVANAGH, 1942), *T. violaceum* (thiamin, pyrimidine and para-aminobenzoic acid; GEORG, 1951), *Gymnoascus reessii*, *Ctenomyces serratus*, *Arachniotus reticulatus* and *Penicillium spiculisporum*.

Group III. Fungi completely heterotrophic for vitamins such as *Trichophyton interdigitale* (thiamin, inositol, pantothenic acid and crude lactoflavin; MOSHER, et al., 1936).

For further reference to vitamin requirements of fungi the review by ROBBINS & KAVANAGH (1942) is recommended.

IV. Trace element requirements.

Table IV summarizes the results of experiments to determine the trace element requirements of the fungi under investigation. A small amount of growth, up to 6.3 mg dry weight was observed in

TABLE IV

Trace element requirements of the fungi tested.

	CONTROL I	CONTROL II	CONTROL III	CONTROL IV	Fe omitted from CONTROL III	only Fe in CONTROL III	Zn omitted from CONTROL III	only Zn in CONTROL III	Mn omitted from CONTROL III	only Mn in CONTROL III	Cu omitted from CONTROL III	only Cu in CONTROL III
Initial pH	6.2	6.0	6.1	5.7	5.75	5.5	5.7	5.7	5.4	5.6	5.6	5.7
MYXOTRICHUM UNCINATUM	6.9	6.5	6.2		6.2	6.3	6.4	6.4	6.4	6.3	6.3	6.3
	83.9	6.3	17.8	—	19.3	16.0	18.5	19.3	19.9	14.6	23.1	8.8
EIDAMELLA DEFLEXA	6.3	6.3	4.4	6.5	4.5	6.1	5.8	4.5	4.5	6.1	5.9	4.7
	43.9	0	69.3	0	67.2	0	0	68.8	59.9	0	0	59.9
GYMNOASCUS REESSII	7.0	6.3	6.1		6.3	6.2	6.1	6.3	6.1	6.3	6.2	6.1
	11.6	6.1	10.7	—	5.6	15.9	11.6	7.9	11.0	6.9	10.7	10.3
CTENOMYCES SERRATUS	7.1	6.6	6.1	6.4	6.6	6.2	6.4	6.3	6.1	6.2	6.2	6.3
	16.7	8.4	8.5	10.8	8.7	5.7	0	6.1	0	0	6.1	15.8
ARACHNIOTUS RETICULATUS	6.8	6.5	6.4	6.5	6.5	6.4	6.4	6.5	6.4	6.5	6.3	6.4
	26.0	6.0	0	10.5	0	10.8	0	7.6	0	10.6	8.0	15.2
PENICILLIUM SPICULISPORUM	6.6	3.5	4.0		3.7	3.4	3.9	3.8	3.8	3.4	3.9	3.6
	59.8	47.6	24.3	—	21.6	26.7	49.6	26.8	22.88	19.6	22.6	17.1

In each block, the top figure represents the final pH, the middle figure indicates the mg of dry weight, the height of the columns indicates the relative amount of growth.

Control I is the untreated complete glucose-asparagine medium; control II is the treated medium lacking trace elements; control III is the treated medium plus iron, zinc, copper, manganese and $Na_2HPO_4.12H_2O$; control IV is the control III minus the sodium phosphate.

the control medium which lacked trace elements (control II). No growth resulted with a control which lacked $Na_2HPO_4.12H_2O$ (control IV) but which contained all the other trace elements. Therefore, it is evident that a considerable amount of phosphate was removed by the $CaCO_3$ treatment which rendered the medium limiting for phosphate. Control III, which supplied all the trace elements, permitted excellent growth of *E. deflexa.*

In these studies, good response was only obtained with *E. deflexa* for which copper and zinc were found to be essential. Either of the two elements alone supported good growth but when they were employed in combination with iron and manganese neither zinc nor copper could support growth. Wherever growth occurred the culture filtrates of *E. deflexa* were found to be acidic, suggesting the presence of an organic acid. MULDER (1938) claimed that copper favored the production of organic acids, while WASSILJEW (1935) stated that *A. niger* formed oxalic acid in zinc media but not in zinc-free cultures.

Copper and zinc have also been reported to be essential for growth of *A. niger* (RAULIN, 1869, STEINBERG, 1919), *A. fumigatus* and *A. oryzae* (ROBERG, 1928), *A. flavus* (MCHARGUE & CALFEE, 1931) and *Trichophyton interdigitale* (MOSHER, et al., 1936).

Eidamella deflexa did not show a response to iron or manganese. CURRIE (1917) found that iron always accelerated the rate of metabolism and growth of *A. niger* when nitrate was the nitrogen source, but no effect was observed when either ammonia or asparagine was employed as the sole source of nitrogen. In the present investigation asparagine was used as the nitrogen source and, therefore, no conclusions on the effect of iron can be made from our studies.

The poor response obtained from the other species might be due to several reasons. *Myxotrichum uncinatum* produced good growth on untreated glucose-asparagine medium. Following treatment of the medium with $CaCO_3$ this species did not grow well. Probably $CaCO_3$ treatment partially removed some unknown but essential factor which then limited normal growth. In every culture flask the quantity of the treated basal medium was equal. Therefore, the quantity of the supposed unknown essential factor was the same throughout. This may explain the more or less equal growth which was observed in all test flasks. Apparently the addition of the various trace elements either had no effect at all, or else the effect was not evident in the absence of that particular factor.

The medium used for *Arachniotus reticulatus*, *G. reessii* and *C. serratus* was limiting with regard to the carbon and nitrogen sources since these fungi do not rapidly utilize glucose and asparagin.

The data for *Penicillium spiculisporum* showed that appreciable growth occurred in the medium which lacked trace elements, and also in the medium which contained iron, manganese and copper but which lacked zinc. It appears that the presence of both copper and manganese is needed for growth of *P. spiculisporum* since no reasonable growth occurred when either of these elements was present alone. FOSTER (1949, p. 254) states that less vigorous growth occurred when essential metallic ions are present singly in a medium. The presence of considerable growth in a medium containing iron, manganese and copper but lacking zinc, supports this assumption. It also suggests that zinc is toxic to this species since growth was suppressed in the presence of zinc. Toxicity of zinc has been reported by LOCKWOOD (1933) for *Penicillium javanicum*, IWANOFF (1904) for *Aspergillus niger* and by TAMMANN & RIENACKER (1927) for *Penicillium glaucum*.

V. Conclusions.

This investigation of the nutritional requirements of certain Gymnoascaceae indicates the following:

1. *Myxotrichum uncinatum* and *Eidamella deflexa* seem more closely related to each other on a nutritional basis than they are to *Arachniotus reticulatus*, *Ctenomyces serratus* and *Gymnoascus reessii*.
2. *Myxotrichum uncinatum* and *Eidamella deflexa* have nutritional requirements more similar to the Aspergillaceae than to the Trichophytoneae.
3. *Ctenomyces serratus*, *G. reessii* and *A. reticulatus* have similar nutritional requirements.
4. *Ctenomyces serratus*, *G. reessii* and *A. reticulatus* have nutritional requirements more similar to those of the Trichophytoneae than to the Aspergillaceae.
5. The nutritional requirements of *Penicillium spiculisporum* are very similar to those of other Aspergillaceae. Therefore, the present investigation supports the separation of this fungus from the Gymnoascaceae.
6. No clear cut physiological evidence is presented which would better support a classification which related the Gymnoascaceae more closely to the Aspergillaceae or to the Trichophytoneae than has been suggested by morphological studies.

SUMMARY

A comparative nutritional study was made of the following fungi: *Arachniotus reticulatus*, *Myxotrichum uncinatum*, *Eidamella deflexa*, *Gymnoascus reessii*, *Ctenomyces serratus* and *Penicillium spiculisporum*. Sodium nitrate, ammonium nitrate, ammonium sulfate, asparagine, glycine, casein hydrolysate and an amino acid mixture were supplied singly as sources of nitrogen. All of the fungi grew on the organic nitrogen sources. The amino acid mixture was the best source for growth of *M. uncinatum*, *G. reessii*, *C. serratus* and *A. reticulatus*. Asparagine was the best source for *E. deflexa* and *P. spiculisporum*, although the latter grew on all 7 sources of nitrogen tested. Of the inorganic nitrogen sources tested sodium nitrate was

assimilated only by *M. uncinatum* and *E. deflexa*. Glucose, fructose, galactose, maltose, sucrose, lactose, raffinose, xylose, dextrin and sodium lactate were the ten carbon sources which were tested. *Myxotrichum uncinatum* failed to assimilate only lactose, and grew best on raffinose, dextrin and sucrose. *Eidamella deflexa* used all carbohydrates but not sodium lactate. *Gymnoascus reessii* either did not grow at all or else grew only poorly on all carbon sources tested. *Ctenomyces serratus* assimilated only fructose. *Arachniotus reticulatus* grew on maltose, fructose and glucose in that order. *Penicillium spiculisporum* used galactose, sucrose, xylose, glucose, maltose, dextrin and raffinose and failed to use lactose and sodium lactate. Thiamin, biotin, inositol, pyridoxine and calcium pantothenate were used in vitamin requirement studies. *Myxotrichum uncinatum* and *Eidamella deflexa* appeared to be autotrophic for all vitamins, *G. reessii* was heterotrophic for biotin and pyridoxine, *C. serratus* for thiamin and *A. reticulatus* for thiamin and pyridoxine. *Penicillium spiculisporum* showed a partial deficiency for pyridoxine and pantothenate. The trace elements which were tested included iron, zinc, copper and manganese. Probably only the results with *E. deflexa* are valid since the carbon and nitrogen sources were inadequate for *G. reessii, C. serratus* and *A. reticulatus*. Zinc and copper were essential for growth of *E. deflexa*. Manganese and copper seemed essential for growth of *P. spiculisporum*, while zinc was toxic.

Literature Cited

Allison, F. E., et al. 1934. Nitrogen fixation studies with fungi and actinomycetes J. Agric. Res. **49:** 1115—1123.

Benjamin, R. K. 1956. A new genus of the Gymnoascaceae with a review of the other genera. Aliso **3:** 301—328.

Bodin, E. 1899. Sur la forme *Oospora* (streptothrix) du *Microsporum* du cheval. Arch. Parasitol. **2**: 362—376.

—1902. Sur le champignon du favus de la souris (*Achorion Quinckeanum*) Arch. Parasitol. **5:** 5—30.

Currie, J. F. 1917. The citric acid fermentation of *A. niger*. J. Biol. Chem.**31:** 15—37.

Czapek, F. 1902. Untersuchungen über die Stickstoffgewinnung und Eiweissbildung der Pflanzen. Beiträge zur Chemischen Physiologie und Pathologie. Band 1, Heft **10—12:** 540—560; Band 3, pp 47—66. 1903.

Dox, A. W. 1910. The intracellular enzymes of *Penicillium* and *Aspergillus* with special reference to those of *P. camemberti*. Bull. U.S.D.A. Bur. Animal Ind. 120.

Emmons, C. W. 1931. Observations on *Achorion gypseum*. Myc. **23:** 87—95.

Delamater, E. C. 1937. *Eidamella spinosa* (Matruchot and Dassonville) refound. Myc. **29:** 572—582.

Dodge, C. W. 1935. Medical Mycology. C. V. Mosby Co., 900 pp.

Duggar, B. M. & Davis, A. R. 1916. Studies in the physiology of the fungi. I. N_2 fixation. Ann. Mo. Bot. Gard. **3:** 413—437.

Foster, J. W. 1949. Chemical activities of fungi. Academic Press, N. Y., 648 pp.

Georg, L. K. 1951. The relation of nutrition to the growth and morphology of *Trichophyton violaceum*. Part I. The vitamins and amino acid requirements of *T. violaceum*. Myc. **43:** 279—309.

Goddard, D. R. 1934. Phase of metabolism of *Trichophyton interdigitale* Priestly. J. Infect. Dis. **54:** 149—163.

GREGORY, P. H. 1935. The dermatophytes. Biol. Rev. Cambridge Phil. Soc. **10:** 208—233.

HAWKER, L. E. 1936. The effect of certain accessory growth substances on the sporulation of *Melanospora destruens* and of some other fungi. Ann. Bot. **50:** 699—717

ITZEROTT, D. 1936. Über die Bedingungen der Stickstoffaufnahme, vor allem der Nitrataufnahme, bei *Aspergillus niger*. Flora **131:** (N.S. 31): 60—86.

IWANOFF, K. W. 1904. Über die Wirkung einiger Metallsalze und einatomiger Alkohole auf die Entwicklung von Schimmelpilzen. Cbl. Bakt. Abt. II, **13:** 139—144.

KUEHN, H. H. 1955. Observations on Gymnoascaceae. I. *Myxotrichum uncinatum* and a new species of *Myxotrichum*. Myc. **47:** 533—545.

—1957. Observations on Gymnoascaceae. IV. A new species of *Arachniotus* and a reconsideration of *Arachniotus trisporus*. Myc. **49:** 55—67.

—1958. A preliminary survey of the Gymnoascaceae. I. Myc. **50** 417—439.

—1959. A preliminary survey of the Gymnoascaceae. II. Mycologia **51:** (in press).

—& CROSBY, P. F. 1959. Growth and nutrition of *Pseudoarachniotus roseus* in submerged culture. Mycopathol. Mycol. Appl. **11:** 109—127.

LATHAM, M. E. 1909. Nitrogen assimilation of *Sterigmatocystis nigra* and the effect of chemical stimulation. Bul. Tor. Bot. Club **36:** 235—244.

LILLY, V. G. & BARNETT, H. L. 1951. Physiology of the fungi. McGraw-Hill Book Co., N. Y., 464 pp.

LOCKWOOD, L. B. 1933. A study of the physiology of *P. javanicum* Van Beyma with special reference to the production of fat. Catholic Univ. Amer. Biol. Ser. No. 13.

McHARHUE, J. S. & CALFEE, R. K. 1931. Effect of manganese, copper, and zinc on growth and metabolism of *Aspergillus flavus* and *Rhizopus nigricans*. Bot. Gaz. **91:** 183—193.

MOSHER, W. A., et al. 1936. Nutritional requirements of pathogenic mold *Trichophyton interdigitale*. Plant Phys. **2:** 795.

MOYER, A. J., MAY, O. E. & HERRICK, H. T. 1936. The production of gluconic acid by *P. chrysogenum*. Zbl. Bakt. 2 Abt., **95:** 311—324.

MULDER, E. G. 1938. Over de beteekenis van koper voor de groei van planten en micro-organismen. Diss., Wageningen.

PURIEWITSCH, K. 1895. Ueber die Stickstoffassimilation bei den Schimmelpilzen. Ber. dtsch. Bot. Ges.**13:** 342—395.

RAPER, K. B. & THOM, C. 1949. Manual of the Penicillia. Williams and Wilkins Co. Baltimore. 875 pp.

RAULIN, J. 1869. Etudes chémiques sur la végétation Ann. Sci Nat. Ser. V. **11:** 93—229.

ROBERG, M. 1928. Ueber die Wirkung von Eisen, Zink und Kupfersalzen auf Aspergillen. Zbl. Bakt., 2 Abt., **74:** 333—370.

1932. Zur Frage nach der Assimilation des molekularen Stickstoffs durch Aspergillen. Zbl. Bakt. 2 Abt., **86:** 466—479.

ROBBINS, W. J. 1937. The assimilation by plants of various forms of nitrogen. Amer. J. Bot. **24:** 243—250.

& KAVANAGH, V. 1942. Vitamin deficiencies of the filamentous fungi. Bot. Rev. **8:** 411—471.

& MA, R. 1945. Growth factors for *Trichophyton mentagrophytes* Amer. J. Bot. **32:** 509—523.

& MCVEIGH, 1946. Effect of hydroxyproline on *Trichophyton mentagrophytes* and other fungi. Amer. J. Bot. **33:** 638—647.

ROSENBAUM, E. 1944. The development and systematic position of *Arachniotus trisporus*. Ann. Mo. Bot. Gard. **31:** 173—202.

SACCARDO, P. A. 1895. Sylloge fungorum. **11:** 437.

SAKAMURA, T. 1930. Die Resorption des Ammonium and Nitratstickstoffs durch *A. oryzae*. Planta Arch. Wiss. Bot. **11:** 765—814.

—1934. Ammonio und Nitratophilie bei *A. oryzae* im besonderen Zusammenhang mit Schwermetallen. J. Fac. Sci. Hokkaido Imp. Univ. Ser. V. **3:** 121—138.

SCHOPFER, W. H. & BLUMER, S. 1940. Recherches sur la répartition de l'hétérotrophie par rapport à l'aneurine chez les champignons. Arch. Mikrobiol. **11:** 205—214.

SCHROEDER, M. 1931. Zur Frage der Assimilation des Luftstickstoffs durch *A. niger* J. Wiss. Bot. **75:** 377—398.

SIDA, K. 1901. Ueber die Assimilation freien Stickstoffs durch Schimmelpilze. Ber. dtsch. Bot. Ges. **19:** 107—115.

STEINBERG, R. A. 1919. A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. Amer. J. Bot. **6:** 330—372.

—1939a. Effects of nitrogen compounds and trace elements on growth of *Aspergillus niger*, J. Agr.ic Res. **59:** 749—763.

—1939b. Growth of fungi in synthetic nutrient solutions. Bot. Rev. **5:** 327—350.

—1941. Sulfur and trace element nutrition of *Aspergillus niger*. J. Agric. Res. **63:** 109—127.

—1942a. Effects of trace elements on growth of *Aspergillus niger* with amino acids. J. Agr. Res. **64:** 455—475.

—1942b. The process of amino acid formation from sugars in *Aspergillus niger*. J. Agric. Res. **64:** 615—633.

—1950. Growth of fungi in synthetic nutrient solutions. 2. Bot. Rev. **16:** 181—228.

STOUT, P. R. & ARNON, D. I. 1939. Experimental methods for the study of the role of copper, manganese and zinc in the nutrition of higher plants. Amer. J. Bot. **26:** 144—149.

TAMIYA, H. 1932. Ueber die Verwendbarkeit von verschiedenen Kohlenstoffverbindungen im Bau-und Betriebsstoffwechsel der Schimmelpilze. Studien über die Stoffwechsel Physiologie von *Aspergillus oryzae*. IV. Acta Phytochim. (Jap.) **6:** 1—129.

—& USAMI, S 1940. Ueber das Wachstum von *Aspergillus oryzae* bei Zugabe der Aminosäuren als alleinige Kohlenstoff- und Stickstoff-quelle. Acta Phytochim. (Jap.) **11:** 261—298.

TAMMANN, G. & RIENACKER, W. 1927. Ueber die Giftwirkung einiger Metalle und Metallegierungen auf Bakterien. Z. anorgan. algem. Chem. **170:** 288—300.

TATE, P. 1929. The dermatophytes or ringworm fungi. Biol. Rev. **4:** 41—75.

VERUJSKY, D. M. 1887. Recherches sur la morphologie et la biologie du *Trichophyton tonsurans* et de *l'Achorion schoenleinii*. Ann. Inst. Pasteur **1:** 369—391.

VOLKONOSKY, M. 1934. Sur la nutrition de quelques champignons saprophytes et parasites. Ann. Inst. Pasteur **52:** 76—101.

WASSILJEW, G. 1935. Ueber biochemische Characterisierung einiger Stämme von *Aspergillus niger* hinsichtlich ihres Säurebildungsvermögens. Biochem. Z. **278:** 226—234.

WENCK, P. R., PETERSON, W. H. & FRED, E. B. 1935. The chemistry of mold tissue. IX. Cultural factors influencing growth and sterol production of *A. fisheri*. Zbl. Bakt., Parasitenk, 2 Abt., **92:** 330—338.

WOOSTER, R. C. & CHELDELIN, V. H. 1945. Growth requirements of *P. digitatum*. Arch. Biochem. **8:** 311—320.

YAMAGATA, S. 1934. Ueber die elementäre Zusammensetzung des Schimmelpilzkorpers. Acta Phytochim. (Jap.) **8:** 107—116.

NUTRITION OF EIDAMELLA DEFLEXA. V. EFFECT OF VARYING THE CONCENTRATIONS OF CERTAIN INORGANIC COMPOUNDS ON GROWTH AND PIGMENT ELABORATION

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(22.III.1960)

Previous papers (KUEHN & CROSBY, 1960a, 1960b, 1960c, 1960d) have established the basic nutritional requirements of *Eidamella deflexa* (BERK.) BENJAMIN. The optimum synthetic medium for growth was found to be as follows: glycine, maltose, 0.1 mg % thiamin, 0.2 mg % biotin and 8 mg % uracil at pH 5.0. This medium also permitted optimum elaboration of a red water-soluble pigment if the initial pH was adjusted to 7.0. The highest concentrations of glycine and maltose tested, 5 % of each, permitted maximum growth. However, somewhat lower concentrations were used routinely. It was found that substitution of glucose, sucrose, soluble starch or sorbitol for maltose permitted approximately equivalent growth if small amounts of certain inorganic salts were included in the medium. However, pigment elaboration in a glycine-maltose supplemented medium was not equalled by substitution of other carbon sources.

It was quite evident that the optimum synthetic medium established in previous work must contain all of the biologically essential inorganic elements in amounts sufficient to support growth. The experiments reported herein were designed to investigate the effects of addition of various concentrations of certain inorganic compounds upon both growth and pigment elaboration. All chemicals were C.P. grade and as such were considered to be contaminated by small amounts of various elements. The data presented in this paper, therefore, show certain inorganic elements are limiting factors in the growth obtained on certain media and that the addition of more of the limiting elements results in greater growth, and in some cases the addition of non-limiting compounds results in depression of growth. However, our primary interest was to study possible stimulation of pigment elaboration by inorganic supplements.

MATERIALS AND METHODS

The strain of *Eidamella deflexa* used throughout these studies was RSA (Rancho Santa Ana Botanic Garden) 57. The experimental procedures were similar to those presented and discussed in the previous papers in this series and were originally developed by KUEHN & CROSBY for *Pseudoarachniotus roseus* (1959). The reader is referred to that paper for further details. In brief, the procedures were as follows. Cultures were grown in duplicate using 100 ml medium in 500 ml Erlenmeyer flasks which were incubated for 5,6 or 7 days on a reciprocating shaker at 28° C. The experimental flasks were inoculated with 2 % of 7—8 day old submerged growth brought up in inoculum flasks containing the same medium as the test medium in each experiment. This procedure should have prevented any great carry-over of inorganic constituents in the inoculum. The mycelium was removed by centrifugation and the dry weight was used as an index of growth. Intensity of pigment production was measured at a 1:100 dilution against a water blank using a Bausch and Lomb Spectronic 20 colorimeter at a wave length of 450 m μ in optically clear 18 mm diam. test tubes. All results reported represent the average of at least 2 replicate flasks. In several instances experiments were repeated one or more times to verify results.

In the majority of experiments the following basal medium was used: 4 % carbohydrate (sucrose or maltose), 1.0, 1.5 or 2.0 % glycine, 0.1 mg % thiamin, 0.2 mg % biotin and 8 mg % uracil, with an initial pH of either 5.0 or 7.0 Different amounts of certain inorganic compounds to be tested were added to this medium.

One experiment was performed using the YpSs medium (EMERSON, 1941) with varying amounts of $MgSO_4.7H_2O$ and K_2HPO_4 and with 1.5 % soluble starch and 0.4 % yeast extract, initial pH 7.0.

Another experiment was performed in which 4 % of either maltose or lactose was used with 1 % peptone or glycine. To this basal medium various amounts of $NH_4MoO_{24}.4H_2O$ or $ZnSO_4.7H_2O$ were added in order to give a final desired concentration by weight of the metal.

RESULTS AND DISCUSSION

Early experimental work with *Eidamella deflexa* was performed using the YpSs medium. Since $MgSO_4.7H_2O$ and K_2HPO_4 were normal constituents of that medium it was decided to establish the approximate optimum concentrations of these two ingredients. The experimental flasks were incubated for 6 days, with an initial pH of 7.0. The results are shown in table I and illustrate the need for small amounts of the salts in question to be included in the YpSs medium.

Several preliminary experiments were performed to establish requirements for inorganic constituents in the glycine-maltose basal medium. A range of concentrations was used to establish the approximate levels to be more critically investigated. In certain instances

TABLE I.

Growth of Eidamella deflexa in a soluble starch-yeast extract medium supplemented with various amounts of K_2HPO_4 and $MgSO_4 \cdot 7H_2O$.

Per cent $MgSO_4 \cdot 7H_2O$	Per cent K_2HPO_4	Final pH	Mycelium weight mg/100 ml
0.05	0.0	7.9	471
,,	0.1	7.4	462
,,	0.2	7.1	438
,,	0.3	7.0	397
,,	0.4	7.0	469
0.0	0.1	7.5	388
0.1	,,	7.5	496
0.2	,,	7.2	514
0.3	,,	5.5	465
0.4	,,	4.1	538
0.0	0.0	5.5	255

selection of the range of concentrations was dictated by the report of NYUKSHA (1957) concerning enhancement of pigmentation in "*Gymnoascus setosus*" by metals. Table II presents the results of a 6-day experiment. The data show that certain concentrations of $ZnSO_4.7H_2O$ and K_2HPO_4 stimulated growth to a degree over the controls. This could indicate that the soluble starch and yeast extract used in the YpSs medium had lower amounts of contaminating potassium, magnesium, phosphate, zinc, and sulfate than did the glycine (aminoacetic acid) and C.P. maltose which were used. How-

TABLE II.

Growth of Eidamella deflexa in a glycine-maltose basal medium supplemented with various inorganic compounds.

Supplement	Concentration	Final pH	Mycelium weight mg/100 ml
Controls	—	6.6	849
K_2HPO_4	0.2 %	7.0	1179
,,	1.0 %	6.4	773
,,	2.0 %	5.3	839
$MgSO_4 \cdot 7H_2O$	1.0 %	5.6	577
,,	2.0 %	4.8	556
$FeSO_4 \cdot 7H_2O$	2.5 mg %	5.2	499
,,	5.0 mg %	5.9	552
,,	10.0 mg %	5.4	512
$CuSO_4 \cdot 5H_2O$	0.25 mg %	6.0	537
,,	0.5 mg %	5.3	518
,,	1.0 mg %	5.3	479
$ZnSO_4 \cdot 7H_2O$	2.5 mg %	5.5	474
,,	5.0 mg %	5.5	727
,,	10.0 mg %	5.2	1041
,,	20.0 mg %	5.2	520

ever, the degree of pigmentation was not appreciably influenced by the various supplements.

Subsequent experiments (1960c) showed that sucrose could be substituted for maltose in the basal medium with no decrease in growth providing certain salts were added. Table III illustrates the growth of *E. deflexa* in a basal medium with sucrose as the source of carbon and with varying concentrations of $MgSO_4 \cdot 7H_2O$ and K_2HPO_4. The degree of pigmentation was approximately the same in all flasks, and thus did not seem to be greatly dependent upon the salts which were tested. Examination of the data in table III revealed that in complete absence of K_2HPO_4 the amount of growth was approximately half the growth at 0.1 %, regardless of the amount of $MgSO_4 \cdot 7H_2O$ present. A 0.2 % concentration of K_2HPO_4 was consistently superior to any other concentration, and the highest mycelium weight resulted with use of 0.2 % $MgSO_4 \cdot 7H_2O$. The conclusion, therefore, was that 0.2 % of each of the salts would approximate the optimum levels.

Table III.

Growth of Eidamella deflexa in a sucrose-glycine medium with various amounts of K_2HPO_4 and $MgSO_4 \cdot 7H_2O$.

		Per cent K_2HPO_4					
		0.0	0.1	0.2	0.3	0.4	0.5
Per cent $MgSO_4 \cdot 7H_2O$	0.0	582*	892	1040	786	773	736
	0.1	483	999	1040	998	674	858
	0.2	456	1258	1151	885	899	808
	0.3	608	971	1109	907	925	835
	0.4	567	1114	1241	987	1005	777

* Mg/100 ml.

Further investigation of the effects of copper, iron and zinc seemed to be warranted. Moreover, the interaction among copper, PO_4, SO_4, iron, zinc, potassium and magnesium was considered worthy of study. A "triangle" experiment using a glycine-sucrose basal medium was designed to accomplish this purope. Figure 1 illustrates the design of the experiment and the concentrations and combinations used for $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$ and $FeSO_4 \cdot 7H_2O$. Three flasks were used for each point on the triangle, but the three flasks differed by the addition of 0.2 % K_2HPO_4 to one, 0.2 % Mg $SO_4 \cdot 7H_2O$ to another and 0.2 % of each to another. There were 63 experimental flasks and two sets of control flasks. Control flasks 64—66 contained 0.2 % each of $MgSO_4 \cdot 7H_2O$ and K_2HPO_4, while control flasks 67—69 had 0.25 mg % $CuSO_4 \cdot 5H_2O$ and 5 mg % each of $ZnSO_4 \cdot 7H_2O$ and $FeSO_4 \cdot 7H_2O$. Of each set of three experimental flasks the flask containing both $MgSO_4 \cdot 7H_2O$ and K_2HPO_4 consistently supported the most growth, the flask with K_2HPO_4 usually supported an amount of growth slightly less, while the flask with only $MgSO_4 \cdot 7H_2O$ had less than half of the growth of either

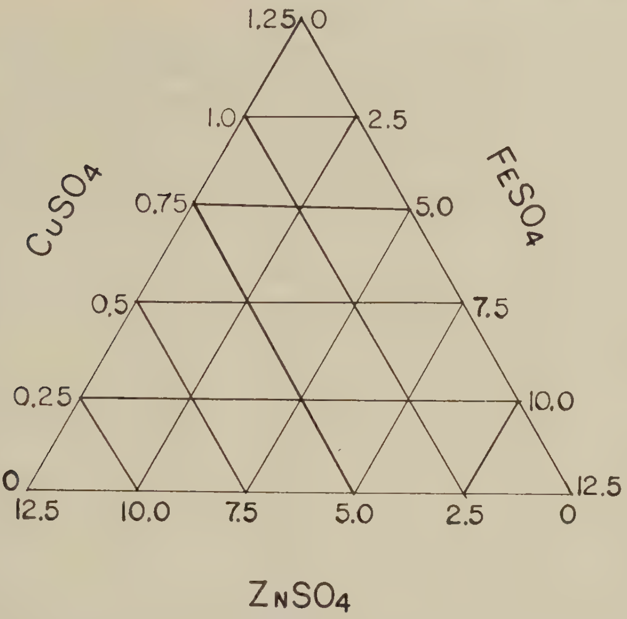

Fig. 1. Diagram illustrating triangle system of varying the concentrations of three salts in a culture medium. Numbers refer to the concentration expressed in mg. per cent.

of the other two flasks. This indicated the seeming lack of requirement for additional $MgSO_4 \cdot 7H_2O$ in a glycine-sucrose medium, and the need for additional K_2HPO_4. However, another explanation would be that K_2HPO_4 was a limiting factor while $MgSO_4 \cdot 7H_2O$ was present in sufficient quantity as contaminants. Then addition of more K_2HPO_4 seemed to stimulate growth. The concentration of copper, iron or zinc did not seem to effect the growth response of $MgSO_4 \cdot 7H_2O$ or K_2HPO_4, nor did the presence of copper, iron or zinc substitute for absence of K_2HPO_4 or $MgSO_4 \cdot 7H_2O$. The degree of pigmentation varied but little throughout the experiment, indicating the independence of pigment elaboration with respect to concentration of the test compounds.

A second "triangle" experiment was devised similar to the first with the exception that 0.2 % each of $MgSO_4 \cdot 7H_2O$ and K_2HPO_4 was included in the basal medium. Two replicate flasks were used for each point on the triangle. Table IV illustrates the results following a 7 day period of incubation. The best growth resulted with the following concentrations: 0.25 mg % $CuSO_4 \cdot 5H_2O$, 0.0 % $ZnSO_4 \cdot 7H_2O$ and 10.0 mg % $FeSO_4 \cdot 7H_2O$, with an increase of about 350 mg over the controls. Too much copper seemed to be quite toxic. Pigmentation did not see to be affected by changes in concentrations of the test compounds.

NYUKSHA (1957) reported that production of a red pigment by

TABLE IV.

Growth of Eidamella deflexa in a sucrose-glycine basal medium with variable concentrations of $CuSO_4 \cdot 5H_2O$, $FeSO_4 \cdot 7H_2O$ *and* $ZnSO_4 \cdot 7H_2O$.

Mg % $CuSO_4 \cdot 5H_2O$	Mg % $ZnSO_4 \cdot 7H_2O$	Mg % $FeSO_4 \cdot 7H_2O$	Final pH	Mycelium weight mg/100 ml
1.25	0.0	0.0	4.6	461
1.00	2.5	0.0	4.5	536
,,	0.0	2.5	4.7	969
0.75	5.0	0.0	4.4	1375
,,	2.5	2.5	4.2	1157
,,	0.0	5.0	5.0	1255
0.50	7.5	0.0	4.4	1421
,,	5.0	2.5	4.4	1457
,,	2.5	5.0	4.3	1213
,,	0.0	7.5	5.1	1366
0.25	10.0	0.0	4.8	1498
,,	7.5	2.5	4.3	1411
,,	5.0	5.0	4.6	1486
,,	2.5	7.5	4.5	1492
,,	0.0	10.0	5.2	1648
0.0	12.5	0.0	4.7	1496
,,	10.0	2.5	4.5	1403
,,	7.5	5.0	4.5	1584
,,	5.0	7.5	4.4	1563
,,	2.5	10.0	4.4	1544
,,	0.0	12.5	4.7	1518
0.0	0.0	0.0	5.1	1294

"*Gymnoascus setosus*" was enhanced by addition of certain concentrations of metals, per cent by weight, to the medium as follows: 0.01—0.02 % molybdenum, 0.005 % zinc, 0.001 % copper, 0.01 % iron and 0.005 % cobalt. KUEHN & CROSBY (1960a) discussed the possibility that the "*G. setosus*" used by NYUKSHA actually represented a strain of *Eidamella deflexa* and at that time they performed the following experiment to determine the effect upon growth and pigmentation of these metals at the concentrations used by NYUKSHA. The medium contained lactose at the carbon source and one of the following as nitrogen sources: glycine, KNO_3, ammonium lactate or peptone. Results showed that under certain conditions zinc, copper and iron enhanced pigmentation but, with one exception, not to the extent reported by NYUKSHA. Zinc was quite effective in stimulating pigmentation in glycine-lactose and peptone-lactose media.

Our present findings in experiments presented in tables II and IV indicated that additional copper, iron and zinc did not affect pigment production in a glycine-sucrose medium. It was decided to test the effect of additional molybdenum and zinc in the optimum maltose-glycine medium as well as in certain other media. The concentrations of molybdenum and zinc were those reported by NYUKSHA to be effective in a peptone-sucrose medium. Following

Table V.

Growth and pigmentation of Eidamella deflexa in various media as influenced by addition of molybdenum or zinc.

Medium	Per cent Molybdenum	Per cent Zinc	Final pH	Optical Density	Mycelial weight mg/100 ml
Maltose-glycine	—	—	5.7	.456	1236
,, ,,	0.01	—	5.8	.658	1109
,, ,,	0.02	—	5.2	.602	1025
,, ,,	—	0.005	5.1	.444	1243
,, peptone	—	—	5.2	.137	1770
,, ,,	0.01	—	4.9	.201	1267
,, ,,	0.02	—	5.6	.222	1336
,, ,,	—	0.005	5.4	.181	1536
Lactose-glycine	—	—	5.4	.456	1236
,, ,,	0.01	—	4.7	.114	395
,, ,,	0.02	—	5.9	.051	197
,, ,,	—	0.005	4.3	.036	192
,, peptone	—	—	5.9	.071	821
,, ,,	0.01	—	5.8	.071	471
,, ,,	0.02	—	5.7	.009	332
,, ,,	—	0.005	5.3	.114	961

a 5 day incubation period the results were those presented in table V. The data show that while molybdenum increased pigmentation in the maltose glycine and maltose-peptone media, growth was inhibited in each medium. However, the stimulatory effect of the metal was slight. Molybdenum decreased both growth and pigmentation in lactose-glycine and lactose-peptone media. Addition of zinc had little effect on either growth or pigment elaboration in the two maltose media. However, in the lactose-glycine medium zinc decreased growth as well as pigmentation, while in the lactose-peptone medium zinc increased both growth and pigmentation slightly.

The present findings of the effect of zinc in lactose media are not in agreement with our earlier findings. However, as stated previously (1960a), although lactose was consistently found to be a good carbon source for growth, the degree of pigmentation in lactose media was quite variable from one experiment to another. The cause for this variability has not yet been elucidated.

Summary

The effect of addition of varying concentrations of certain inorganic compounds in selected media upon growth and pigment elaboration of *Eidamella deflexa* (Berk.) Benjamin was studied in submerged culture. Preliminary experiments were performed to establish approximate optimum ranges of certain inorganic compounds. Further investigations in a glycine-sucrose basal medium indicated that the following range of concentrations stimulated growth to the greatest extent: 0.2% $MgSO_4 \cdot 7H_2O$, 0.2% K_2HPO_4, 0.25 mg%

$CuSO_4 \cdot 5H_2O$, 0,0% $ZnSO_4 \cdot 7H_2O$ and 10.0 mg % $FeSO_4 \cdot 7H_2O$. The degree of pigment production did not seem to be affected by different concentrations of these compounds.

The effects of additional molybdenum and zinc upon growth and pigmentation were studied in the following media: lactose-peptone, lactose-glycine, maltose-peptone and maltose-glycine. Molybdenum increased pigmentation slightly in maltose-glycine and maltose-peptone media, but inhibited the amount of growth. Molybdenum decreased both growth and pigmentation in the two lactose media. Zinc had little affect on either growth or pigment elaboration in the two maltose media. However, in the lactose-glycine medium, zinc decreased both growth and pigmentation while in lactose-peptone media zinc slightly increased growth and pigmentation.

References

Emerson R. 1941. An experimental study of the life cycles and taxonomy of *Allomyces*. Lloydia, **4**, 77—144.

Kuehn, H. H. & Crosby, P. F. 1959. Growth and nutrition of *Pseudoarachniotus roseus* in submerged culture. Mycopath. et Mycol. Appl., **11**, 109—127.

Kuehn, H. H. & Crosby, P. F. 1960a. Nutritional studies of *Eidamella deflexa*. I. Carbon-nitrogen assimilation and some growth factor requirements. Mycopath. et Mycol. Appl.,**12**, 145—155.

Kuehn, H. H. & Crosby, P. F. 1960b. Nutrition of *Eidamella deflexa*. II. Development of a synthetic medium for optimum growth and pigmentation. Appl. Microbiol., **8**, 133—135.

Kuehn, H. H. & Crosby, P. F. 1960c. Nutrition of *Eidamella deflexa*. III. Further studies on carbon and nitrogen assimilation and pigment synthesis. Appl. Microbiol., **8**, 148—151.

Kuehn, H. H. & Crosby, P. F. 1960d. Nutrition of *Eidamella deflexa*. IV. Rate of utilization of sugar and amino nitrogen compared with rate of growth and pigment elaboration. Mycologia (manuscript submitted, not yet accepted).

Nyuksha, Y. P. 1957. A physiological study of the fungus *Gymnoascus setosus* Eidam. II. Mikrobiologiya, **26**, 309—315.

OBSERVATIONS ON GYMNOASCACEAE. VIII. A NEW SPECIES OF ARTHRODERMA.

BY

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(14.IV.1960)

CURREY (1854) illustrated and briefly described a fungus which he did not recognize and which he tentatively regarded as new to science. He considered it to be "almost identical" with *Trichoderma*, but stated that it differed in the jointed nature of the hyphae around the spores and in the color of the spores, which were "brilliant yellow" The fungus occurred on dead leaves and sticks lying on the ground in a wooded area in moist, almost muddy, conditions. He did not fully describe this fungus, although if it was described as a new genus in the future CURREY suggested that *Arthroderma* would be an appropriate name. BERKELEY (1860) followed the suggestion of CURREY and described this fungus as a new genus and species, *Arthroderma curreyi*.

BENJAMIN (1956) reviewed the subsequent errors of mycologists who came to regard *A. curreyi* as representing the perfect stage of *Ctenomyces serratus* EIDAM. These two species are now regarded as distinct representatives of the Gymnoascaceae (KUEHN, 1958).

In the spring of 1954 the writer observed fungus growth consisting of white tufts on some material in a moist chamber. This material, feathers of a robin, had been collected in the vicinity of Urbana, Illinois. When the tufts of fungus growth on the feathers were examined under the microscope it was apparent that they represented the ascocarps of a Gymnoascaceous fungus, similar to, if not identical with, *Arthroderma curreyi*. The fungus was isolated in pure culture and grew well on YpSs, Sabouraud's and potato dextrose media. However, the ascosporic stage was not observed in pure cultures.

Within a few weeks another strain of this same fungus was isolated from an owl pellet which also had been placed in a moist chamber. This second strain also grew well in pure culture but did not form ascospores on any media upon which it was cultured. Since 1954 these two strains have been maintained in culture, with no apparent production of the ascosporic stage.

Recently, DAWSON & GENTLES (1959) described the perfect stage of *Keratinomyces ajelloi*. Their illustrations and description were such to suggest that they possibly were concerned with a species of *Arthroderma*. Thus, it appeared possible that *K. ajelloi* was the imperfect stage of a new species of *Arthroderma*, and in a personal communication I suggested this theory to DAWSON. She kindly sent me mating strains of her isolates, and an examination of that material revealed nothing to alter my early impression that *K. ajelloi* was an *Arthroderma*. However, it is not my intention to elaborate upon further studies of DAWSON'S strains at this time.

Nevertheless, the stimulation afforded by the report of DAWSON & GENTLES caused a re-examination of available cultures of *Arthroderma* and related forms. During this study material from the original cultures (inoculated April, 1954) of the two supposedly non-fruiting strains from robin feathers and owl pellet was examined and was found to contain abundant ascospores. New sub-cultures transferred from the original cultures also proved to be ascosporic even after repeated transfer. Although the characteristics of the perfect stage of these two strains seemed to be identical to those of *Arthroderma curreyi*, they could be differentiated from *A. curreyi* on the basis of the accessory spore phase. Based on such differences a new species of *Arthroderma* is proposed, represented by the two strains from Illinois.

Arthroderma tuberculatum n. sp. [1]

Cleistotheciis globosis aut sub-globosis, aggregatis, saepe plus aut minus continuis, cremeo-albis ad cremeo-luteis, 435—970 μ diam. appendiculis inclusis. Hyphis peridii hyalinis, septatis, crasso-parietalibus, asperulatis, cum cellulis biscoctiformibus; cellulis asymmetricis vel symmetricis, elongatis, ca. 4.0 μ latis ad centrum, 6.6—7.0 μ latis ad fines, 10—27 μ longis; appendiculis non-numerosis, angustis, aequalibus, hyalinis, septatis, appendiculis cellularum peridi terminalibus, spiralibus, stricte volutis 10 μ latis, 60 μ longis. Ascis hyalinis, ovoideis, 3.9—4.7 X 4.4—5.5 μ, octosporis. Ascosporis luteis, compresso-oblatis, 1.1—2.0 X 2.5—3.0 (3.5) μ, levibus. Aleuriosporis cremeis, lateralibus raro terminalibus, pedicellatis, semi-sessilibus vel sessilibus, subglobosis, ovoideis raro ellipticis, 8.8—14.3 X 13.2—18.7 (24.2) μ, in aestate tuberculiformibus vel papilliformibus.

Cleistothecia spherical to sub-spherical, creamy-white to creamy-yellow, distinct but often clustered into large aggregates, 435—970 μ diam. appendages included; each ascocarp with one ascigerous mass forming a yellow spherical central region about 2/3 the diam. of the ascocarp. Peridium composed of a network of branched, anastomosed septate, almost hyaline hyphae composed of thick-walled, densely

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[1] The writer acknowledges with appreciation the assistance of Dr. TIBOR BENEDEK in the preparation of the Latin Description.

asperulate, usually dumb-bell shaped cells; cells symmetrical or asymmetrical, elongate, about 4.0 μ wide at narrow waist and 6.6—7.0 μ wide at the enlarged ends, and 10—27 μ long. Infrequently, peridial hyphae terminating in a slender, short, curved, smooth hyphal segment, or terminating in a thin - walled, hyaline, septate, spiral appendage coiled in a spring-like manner with 2—3 turns measuring about 10 μ wide and up to 60 μ long; peridial hyphae usually not terminating in appendages. Asci hyaline, ovoid, 3.9—4.7 X 4.4—5.5 μ, 8-spored, the wall evanescent, ascospores conglomerate for only a short time, then separating. Ascospores bright yellow, smooth, flattened-oblate, often with noticeably flattened sides, 1.1—2.0 X 2.5—3.0 (3.5) μ. Imperfect phase represented by cream colored, lateral or rarely terminal, pedicellate, short-pedicellate or sessile, subglobose, ovoid or rarely elliptical aleuriospores measuring 8.8—14.3 X 13.2 —18.7 (24.2) μ and borne singly, covered at maturity with tuberculations or papillations of variable size and shape; pedicels 2.2 μ diam., and 0.3-12.0 μ long; aleuriospores numerous on aerial hyphae. Racquet mycelium present. Vegetative hyphae hyaline, 2.2—5.0 μ diam.

Colonies on YpSs agar in 7 days at 28° C 2—3 cm. diam., more or less restricted, initially hyaline, then tawny and dense or thick; abundant aleuriospores present; minute white cottony tufts present, representing fruiting areas of young ascocarps; reverse in shades of pale yellow; colonies odorless. In 16 days colonies attain 5—6 cm diam., mostly creamy to tawny, more or less restricted and low growing, but with cottony, white, pleomorphic overgrowths rather general in certain areas of the colony; colorless, small droplets of exudate infrequently present; ascogenous areas of minute, white cottony tufts scattered, not representing the dominant colonial character, but easily distinguished macroscopically; colony not furrowed; faint, slightly moldy to sweet or yeasty odor present; reverse in creamy to buff shades. In 21 days faint moldy-sweet odor remaining; colonies 6—7 cm diam., and similar to the 16 day old colony except ascogenous tufts not white, but similar to and blending with cream colored colony; white, pleomorphic overgrowths present; ascocarps not abundant but with many mature ascospores. No change in appearance of 28 day old colony.

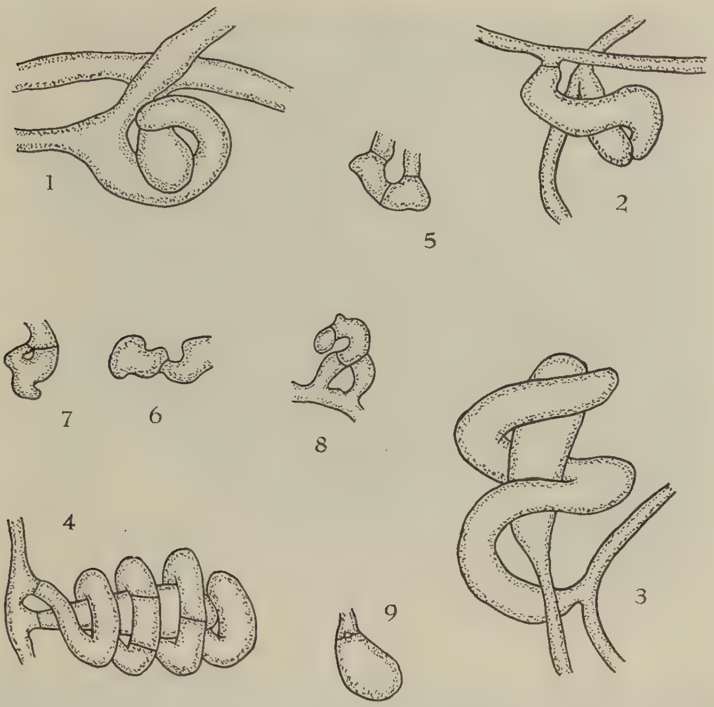
Colonies on Czapek's medium in 7 days at 28C 2—3 cm diam., very sparse and thin, colorless, aerial growth lacking; aleuriospores absent. In 21 days colonies cover the surface of medium in Petri dish, thin, no aerial growth; surface mycelium consisting of aleuriospore-bearing hyphae; perfect stage completely absent.

Colonies on potato dextrose agar at 28 C in 7 days 2—3 cm diam., with many minute, white tufts; colony thinner than on YpSs, with tawny shades lacking; earthy or moldy odor pronounced; reverse the color of the medium. In 16 days colonies almost covering surface of the medium, 7—8 cm diam.; colonies white and thin, with white tufts representing aleuriospore-bearing hyphae; odor not pronounced; reverse colorless. In 21 days colonies covering surface of 9 cm diam. Petri dish; colonies white and thin, reverse colorless; odor faint; ascogenous tissue absent. In 30 days perfect stage absent.

Colonies on Sabouraud's maltose agar in 7 days at 28 C 2—3 cm diam., appearing as on YpSs medium, hyaline, then tawny;abundant aleuriospores present; infrequent white cottony ascogenous tufts present; growth more or less restricted and dense; odor absent; reverse distinctly yellow, more so than on YpSs medium. In 16 days colonies 5—6 cm diam., as on YpSs agar except slightly less pleomorphic growth present; moldy-sweet odor as on YpSs medium; colony restricted and thinner than on YpSs; reverse often streaked with yellow and, in general, creamy-yellow in color. In 21 days colonies 6—7 cm diam., similar in appearance to 16 day colony, and also similar in appearance to colony on YpSs medium except for the yellow color reverse and for less prevalent pleomorphism; perfect stage not abundant; no mature ascospores present. Ascospores present in 29 day old colonies.

The type strain, isolated from robin feathers, has been deposited in the culture collection of the Northern Utilization Research and Development Division, Peoria, Illinois.

Development of ascospores took place in the following manner. The gametangial initials, of which there were few per colony, consisted of slender, lateral branches which arose on the same (Fig. 4) or different parent hyphae (Fig. 1—3). The slender ascogonium coiled about the slender, straight, club-shaped antheridium in a tight (Fig. 4) or loose (Fig. 3) coil. The initials were delimited from the parent hypha by septa (Fig. 4) and soon thereafter the ascogonium became divided into several cells by formation of cross walls (Fig.4). From these cells bulges appeared (Fig. 5) which soon developed into short ascogenous hyphae. Croziers were produced on the ascogenous hyphae but these grew out to form secondary croziers (Fig. 6—8) and no asci were formed at this early stage. Croziers were detected in 9 day old cultures incubated on YpSs medium at 28 C. At this time cleistothecia had become delimited, however, although the peridial hyphae were distinct, the dumb-bell shaped cells were not mature. Each developing ascocarp had only one pair of gametangial initials within it, so that all ascospores in one cleistothecium originated from one pair of initials. Cleistothecia from 9 day old cultures enclosed tightly packed, twisted or coiled ascogenous hyphae and many croziers were in evidence. As the ascogenous hyphae elongated with successive crozier formation, the peridial hyphae became differentiated from vegetative hyphae located at random in the vicinity of the gametangial coil. After about one week of further growth of ascogenous hyphae and enlargement of the cleistothecium, asci were formed from penultimate cells of croziers in great numbers throughout the central areas within the cleistothecium. As the ascospores matured this central area became lemon yellow in color as seen under the microscope.



Figs. 1—9. *Arthroderma tuberculatum*. 1. Origin of gametangia. A slender lateral branch, the ascogonium, coiling about the shorter antheridium which arises as a lateral branch from another hypha. 2—3. Later stages in gametangial relationship. 4. Gametangia arising from the same parent hypha. The ascogonium has become septate. 5. Early stage in the formation of ascogenous hyphae from cells of the ascogonium. 6—8. Stages in development of croziers and their growth into secondary croziers. 9. Early stage in the formation of an ascus. All figures X 1727.

Aleuriospores usually are so short pedicellate as to appear sessile (Fig. 14). Young, immature chlamydospores are smooth-walled, but as they mature they develop decidedly rough walls within 9 days on YpSs agar at 28 C. Germination of the aleuriospores was observed by making dilution plates on malt extract agar. Within 18 hours almost all of the aleuriospores had germinated, with one or two germ tubes per spore. The pattern of origin of the germ tubes was quite variable with the tubes originating on opposite ends of the spore, or close together toward one end. In the 18 hour old cultures germ tubes averaged about 20 μ long, and were branched one or more times. The clavate aleuriospores characteristic of *Arthroderma curreyi* were never encountered in either strain of *A. tuberculatum*.

Asci and ascospores of *Arthroderma curreyi* RSA 186 were similar in size, shape and color to those of *A. tuberculatum*. However, ascocarps of *A. curreyi* were usually smaller and rarely, if ever,

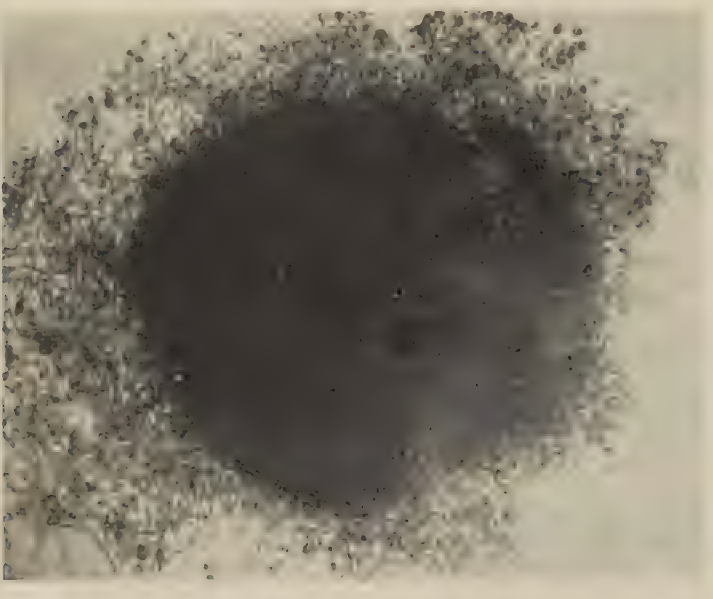


Fig. 10. Mature ascocarp X 143.

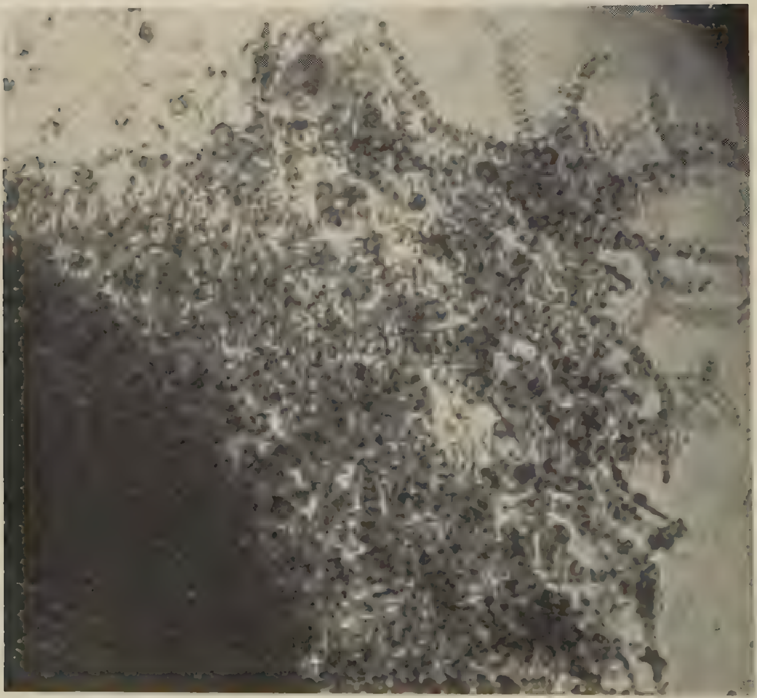


Fig. 11. Peridial hyphae. X 158.

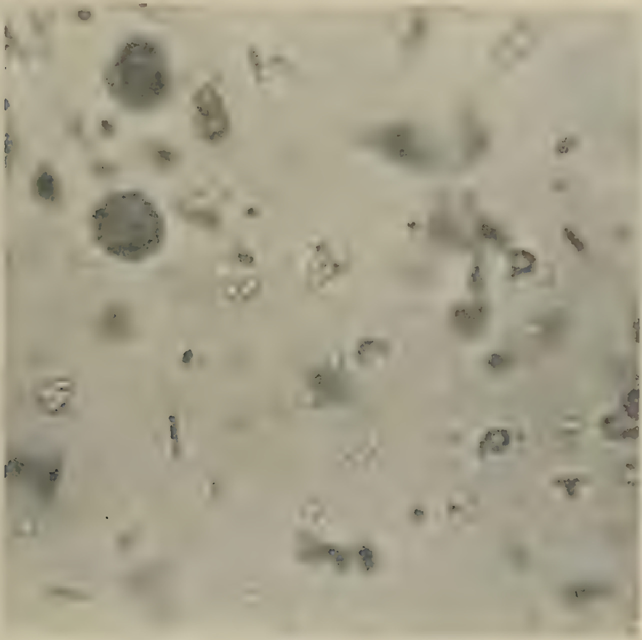


Fig. 12. Ascospores. × 833.

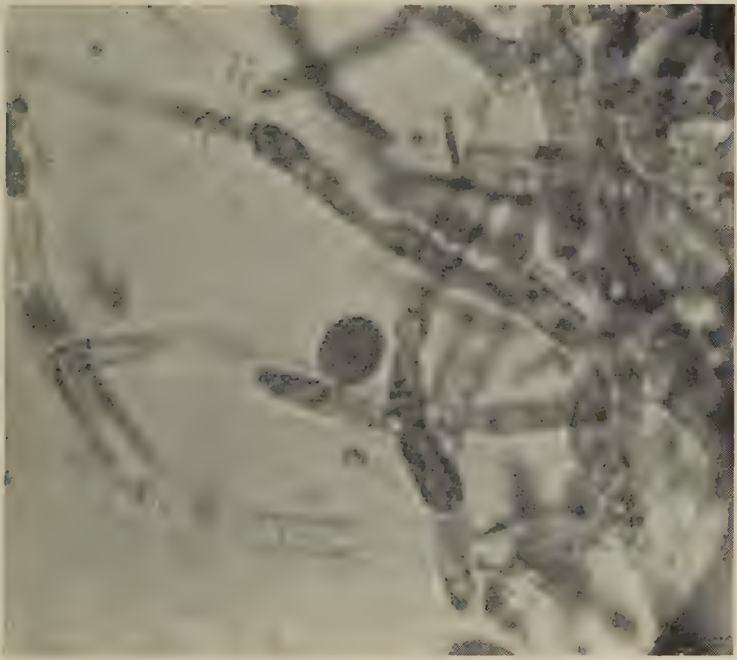


Fig. 13. Young sessile aleuriospore. × 800.

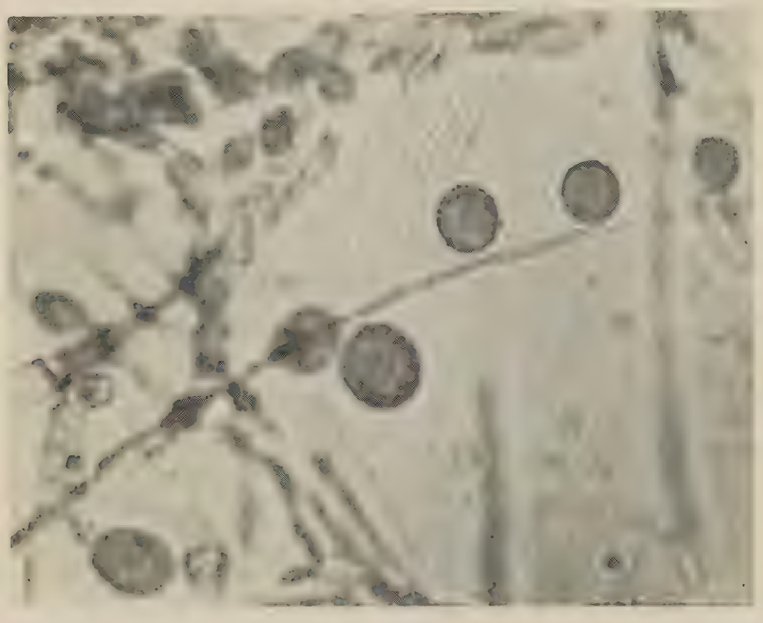


Fig. 14. Immature aleuriospores. × 800.

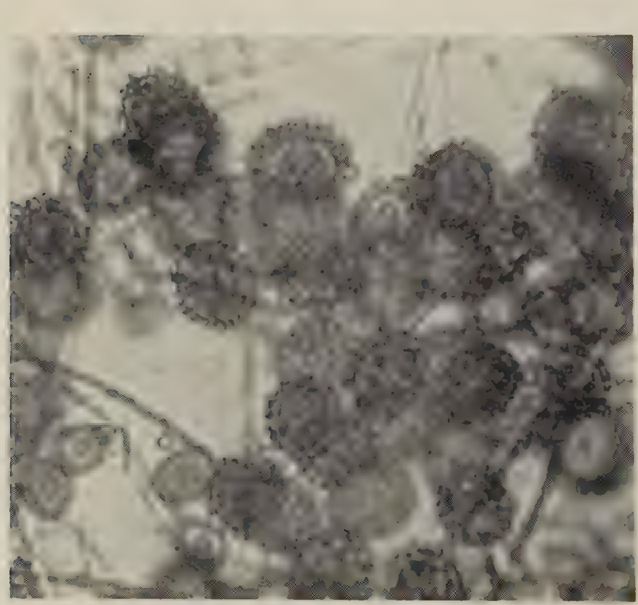


Fig. 15. Mature aleuriospores. × 800.

exceeded 600 μ diam. A culture obtained from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands, as *Ctenomyces serratus*, proved not to represent *C. serratus*. It actually was another strain of *A. curreyi*. This strain did not differ from *A. curreyi* in any important particular. Both cultures formed clavate aleuriospores in abundance but never formed the tuberculate aleuriospores so characteristic of *A. tuberculatum*.

SUMMARY

A new species of *Arthroderma*, *A. tuberculatum*, is described and illustrated. This new species is represented by two strains isolated from feathers of a robin and from an owl pellet. Although *A. tuberculatum* is similar to the only other known species of the genus, *A. curreyi*, with regard to the characteristics of the perfect stage, the two species may easily be distinguished by virtue of the accessory spore forms. *Arthroderma curreyi* forms small aleuriospores while *A. tuberculatum* produces large, tuberculate aleuriospores. The morphological development of the ascocarp is presented along with other diagnostic characteristics. Asci are formed through the intervention of croziers.

LITERATURE CITED

BENJAMIN, R. K. (1956) A new genus of the Gymnoascaceae with a review of the other genera. Aliso **3**: 301—328.

BERKELEY, M. J. (1860) Outlines of British Fungology. Lovell Reeve, London. 442 pp.

CURREY, F. (1854) On two new fungi. Quart. J. Microscop. Sci. **2**: 241—242.

DAWSON, C. O. and GENTLES, J. C. (1959) Perfect stage of *Keratinomyces ajelloi*. Nature **183**: 1345—1346.

KUEHN, H. H. (1958) A preliminary survey of the Gymnoascaceae. I. Mycologia **50**: 417—439.

A COMPARATIVE STUDY ON THE EFFECT OF COTTON DUST AND 2, 4-D ON THREE PATHOGENIC FUNGI

by

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(26.II.1960)

1. Introduction

Cotton is the main crop in Egypt, and the economical prosperity of the country depends largely on its final crop yield. One of the most serious problems, which encounter cotton cultivators in Egypt, is the attack of its leaves by a serious destructive insect "*Prodinia litura*". In the last years, a method has been adopted to avoid the losses due to this insect disease by dusting with cotton dust. The cotton dust consists of a mixture of a filler and active insecticides in the following ratios: 40 % sulphur, 3 % Gama isomer of benzene hexachloride designated as "BHC" and 10 % Dichlorodinitrotrichloroethane designated as "DDT". During the season of cotton cultivation in Egypt through the year 1951, more than 1.6 millions kilograms of cotton dust were applied to an area of about 150000 acres. A great portion of the insecticidal mixture falls into the soil during and after dusting, and this will become more accumulated in the soil with continual dusting in successive years. Two insecticidal components of cotton dust, namely BHC and DDT, were recorded by many investigators to have an accumulative effect in the soil and to be persistent (Smith, 1948 and Fleming, 1949) and that their residues are injurious to some plants and soil microorganisms. The present work has been subsequently carried out in an attempt to elucidate the vigour responses of some soil-borne fungal pathogens to the presence of cotton dust, as a whole mixture and as individual insecticidal components. The experimental pathogenic fungi chosen are: *Fusarium solani* and *Sclerotium bataticola* causing cotton diseases and *Botrytis fabae* responsible for a chocolate-spot disease of broad beans in Egypt. The effect of cotton dust, as well as its individual insecticidal components, on the vigour responses of host seedlings and host-parasite relationship will constitute the subject matters of other papers. The same sequence of experiments has been carried out to study the effect of the herbicide 2,4-Dichlorophenoxyacetic acid, commonly known as 2,4-D.

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Accordingly, comparative studies have been carried out to test the effect of cotton dust and its insecticidal components — as well as the herbicide 2,4-D on the mycelial growth and conidial germinative potentialities of the three experimental fungi. Different concentrations were used to represent different degrees of accumulation in the soil.

Cotton dust has been applied either as a whole mixture, as individual insecticidal components, or as combinations of its various constituents. The possible effect of the filler — present in the commercial cotton dust — has been also evaluated by comparing the mycelial growth of each individual fungus on the commercial cotton dust on one hand and on a prepared mixture containing only the actual insecticidal components, on the other hand.

DDT was recorded to have no or slight toxic effect on fungi. WILSON & CHOUDHRI (1946) recorded that DDT — in a dose far in excess of that required in practice for insect control — exerted no adverse effect on: *Aspergillus niger, A. oryzae,* three *Penicillia* spp., six unidentified fungi, and three actinomycetous members; this has been proved both in soil and in culture. On the other hand, MCFARLAND (1946) showed that it has only slight toxic effect on some wood-destroying fungi. Heavy applications of BHC, up to 82 pounds per acre, were proved to have no harmful effect on the population of soil microorganisms (WILSON & CHOUDHRI, 1948). In culture, however, they reported that its retarding effect, on fungal and bacterial growth, was limited to the first twenty four hours. Its presence was found, on the other hand, to suppress the growth of algal cells. They also proved that the delta isomer is the only BHC isomer responsible for the delay of fungal growth.

A controversy exists, however, with regard to the effect of 2, 4-D, or its derivatives, on growth of fungi. A concentration — up to 0.8 % had no apparent effect on the growth of *Penicillium notatum* and two other fungi (STEVENSON & MITCHELL, 1945). Similarly, LEWIS & HAMNER (1946) found that 2, 4-D applied at normal rates for killing weeds, had no appreciable effect on soil microorganisms and soil-borne plant pathogens. On the other hand, other investigators recorded its toxic effect to fungal conidial germination and mycelial growth. GUISCAFRE-ARRILLAGA (1948) stated that concentration from 0.1 % to 0.3 % were inhibitory for *Penicillium digitatum* conidial germination; the fumes of its ethyl ester resulted in stopping the development of germ tubes. Similarly, WEI & LING (1949) recorded its retarding effect on spore germination of many fungi at different pH values. IBRAHIM (1951) found that *Puccinia graminis avenae* spores failed to germinate in a concentration exceeding 50 p.p.m. of 2,4-D butyl ester. In a similar manner, *Puccinia coronata* uredospores were inhibited at high concentrations of the hormone (PETURSON 1951). BEVER & SLIFE (1948) studied the inhibitory effect of 2,4-D derivatives on the mycelial growth of *Giberella zeae, Helminthosporium victoriae* and *Pythium debaryanum.* They stated that the experimental concentrations (i.e. 250, 500, 1000 and 2000 p.p.m. of the active acid) of the sodium salt and amine ester, killed *Pythium* while the butyl ester retarded its growth in proportion to dilution. On the other hand, *Giberella* or *Helminthosporium* growth was retarded in proportion to dilution of all 2,4-D derivatives used. GARBER, SCHAAL & FULTS (1951) reported that increasing concentration of pentachlorophenoxyacetic acid in culture media—up to 2000 p.p.m. proportionally inhibited the growth of *Streptomyces scabies* and two forms of *Penicillium notatum.* The action, with regard to *Streptomyces,* was fungistatic at concentrations up to 500 p.p.m. and probably fungicidal at 1000 p.p.m. or more. HSIA & CHRISTENSEN (1951) reported that 2,4-D inhibited the growth of *Helminthosporium sativum.* This fungus showed genetic variation; some variants were proved to be more tolerant to the chemical but not more pathogenic than parent.

II. MATERIAL AND METHOD

The three experimental pathogenic fungi are: *Fusarium solani* and *Sclerotium bataticola* isolated from diseased cotton plants and *Botrytis fabae* isolated from chocolate-spotted bean leaves. The insecticedal and herbicidal chemicals were kindly supplied by the Imperial Chemical Industries and Plant Protection Ltd. These are:

Agrocide cotton dust (40 sulphur: 10 DDT: 3 gamma isomer of BHC).
Italian sulphur.
BHC D. 929 (13 % gamma isomer).
DDT pure.
Pure sodium salt of 2,4-D.

The effect of cotton dust was studied, either as a whole mixture, individual components or combinations of each two components so far as the fungal mycelial growth and conidial germination are concerned; the criteria applied are: mycelial lateral spread and dry weight as well as conidial germinative potentiality. Mycelial lateral spread was measured on an agar medium, while mycelial dry weight on a liquid medium; conidial germination was tested by the hanging — drop method using Van Tieghem cells. In case of solid medium, the calculated amount of the experimental chemical, is mixed with sterilized Dox' agar medium, shaken well and distributed in

Petri dishes. Solidified medium was then inoculated with standardized mycelial disc — incubated at 25° C — and the lateral spread, expressed as total diameter, was measured daily for 10 days. The same sequence was adopted, using Dox' liquid without the addition of agar, for measuring the mycelial dry weight. Instead of plates, 25 ccs Dox' liquid — either containing or devoid of the experimental chemical was inoculated in a 150 ccs conical flask with standardized mycelial disc and incubated at 25° C for 10 days. Then mycelial mats were filtered off, washed with sterilized water for several times, dried at 80° C until a constant weight was maintained.

III. Experimental Results

A. Effect of Cotton dust

Comparisons of relative mycelial lateral spreads of either *Fusarium solani* or *Sclerotium bataticola* (Table I) as influenced by a similar concentration viz., 100 and 10,000 p.p.m. of commercial and prepared cotton dust, have demonstrated that the filler in the former could be considered inert so far as the fungal growth is concerned.

Table I

Relative mycelial lateral spreads of *Fusarium* and *Sclerotium*, expressed as total diameter (in mms), on Dox' agar containing 100 p.p.m. or 10000 p.p.m. of either prepared (P) or commercial (C) cotton dust, the non-insecticidal filler has been completely excluded in the prepared cotton dust.

Time (in days)	*Fusarium*				*Sclerotium*			
	100 p.p.m. (Cotton dust)		1000 p.p.m. (Cotton dust)		100 p.p.m. (Cotton dust)		1000 p.p.m. (Cotton dust)	
	(C)	(P)	(C)	(P)	(C)	(P)	(C)	(P)
1	7	7	7	7	7	7	7	7
2	10	11	7	7	18	18	7	7
3	18	18	8	9	42	45	8	8
4	30	29	9	10	72	74	9	9
5	36	37	12	12	—	—	10	10
6	45	47	15	15	—	—	11	11
7	55	57	17	18	—	—	12	12

(*i*) *Effect of cotton dust as a whole*:

A study has been made of the mycelial growth-responses, of the three experimental fungi, to the following concentrations of commercial cotton dust added either to Dox' agar or to Dox' liquid:

100, 500, 2500, 5000 and 10,000 p.p.m.

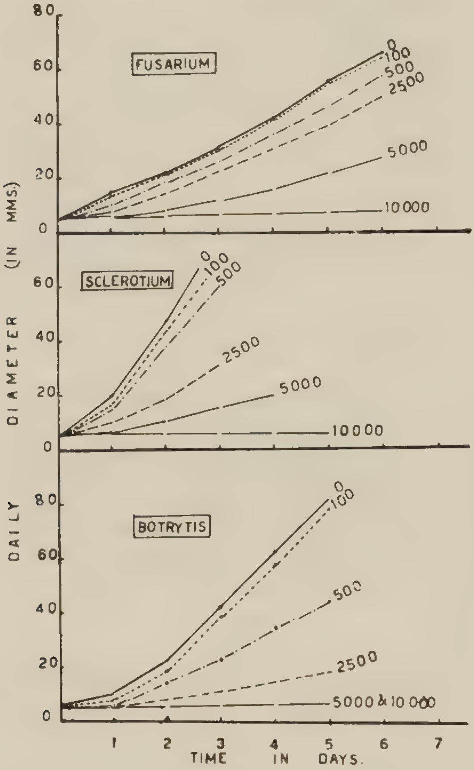

Fig. 1. Growth curves of the three fungi grown on Dox' agar containing varying concentrations of cotton dust.

Growth curves of the three fungi (Fig. 1) have shown gradual depression with increasing concentration of cotton dust. The lowest experimental concentration (i.e. 100 p.p.m.) does not cause an appreciable decrease in the mycelial spread of any one of the three fungi, while fungal spread becomes distinctly very slow at 5000 p.p.m. cotton dust concentration. At 10,000 p.p.m., the mycelial growth of either *Fusarium* or *Sclerotium* seems to be completely inhibited whereas that of *Botrytis* is considerably suppressed.

The mycelial dry weights have been similarly determined on Dox' liquid either alone or with the addition of varying concentrations of cotton dust (Fig. 2). The percentage decrease in weight is also indicated in comparison with that on pure Dox' liquid (Table II). At 100 p.p.m. *Botrytis* shows the highest percentage decrease, followed by *Sclerotium* and the least is *Fusarium*. Such higher sensitivity of *Botrytis* growth is also maintained at 500 and 2500 p.p.m. concentrations. On the other hand, the mycelial

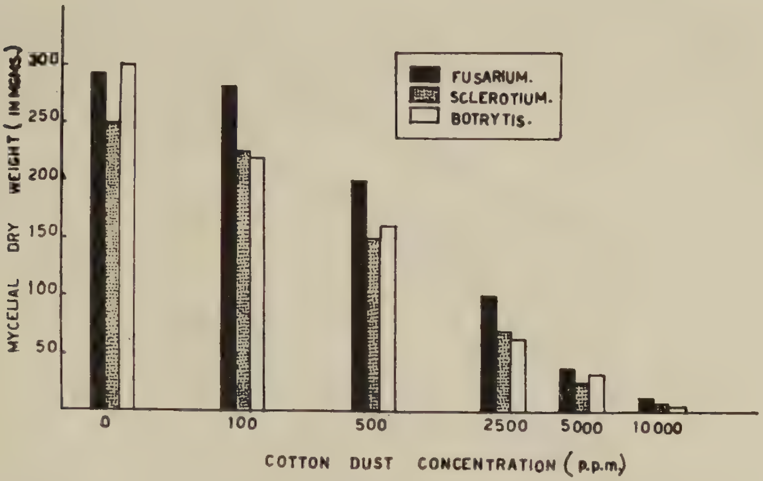

Fig. 2. Mycelial dry weights on Dox' liquid containing varying concentrations of cotton dust.

growths of the three fungi are more or less equally suppressed at 5000 p.p.m. and nearly ceases at 10,000 p.p.m.

(*ii*) *Effect of individual insecticidal components of cotton dust.*

In an attempt to evaluate the individual effect of different insecticidal components of cotton dust, for suppressing the mycelial growth of each one of the three fungi, the highest experimental cotton-dust concentration (i.e. 10,000 p.p.m.) has been chosen. This cotton dust concentration contains: 4000 p.p.m. sulphur, 1000 p.p.m. DDT and 2300 p.p.m. BHC.

Growth curves of the three fungi, in response to cotton dust or its individual constituents (Fig. 3), show that sulphur component causes only a slight depression in mycelial lateral spread in comparison with that of the control. DDT comes next to sulphur with

Table II.

Relative mycelial dry weights (in mgms) — of either *Fusarium, Sclerotium* or *Botrytis* in response to Dox' liquid and to the same medium containing varying concentrations of Cotton dust; percentage decrease in mycelial weight is indicated in comparison with that on pure Dox' liquid.

Experimental fungus	*Mycelial dry weight (in mgms)*						*Percentage decrease*				
	0 (p.p.m.)	100 (p.p.m.)	500 (p.p.m.)	2500 (p.p.m.)	5000 (p.p.m.)	10000 (p.p.m.)	100 (p.p.m.)	500 (p.p.m.)	2500 (p.p.m.)	5000 (p.p.m.)	10000 (p.p.m.)
Fusarium	292	282	200	100	37	12	3 %	32 %	66 %	87 %	96 %
Sclerotium	250	225	150	70	25	7	10 %	40 %	72 %	90 %	97 %
Botrytis	300	220	160	62	33	5	27 %	47 %	79 %	89 %	98 %

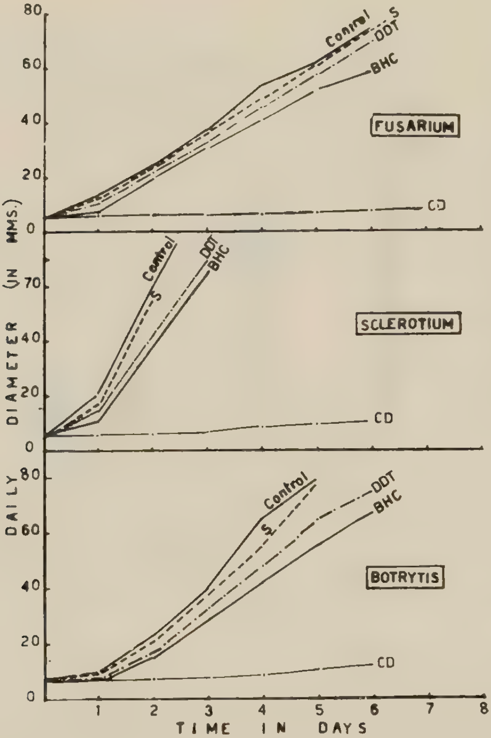

Fig. 3. Growth curves of the three fungi grown on Dox' agar containing 1% cotton dust concentration or the corresponding concentration of each insecticidal component.

regard to its more depressing effect. Its effect is, however, less pronounced in case of *Fusarium*, than in *Sclerotium* and *Botrytis*. The highest depression in mycelial lateral spread is achieved by the BHC constituent (Gammexane); this depressive effect is even very low in comparison with that induced by cotton dust as a whole, which causes an almost complete cessation of mycelial growth. Thus, no individual component has been found which causes a depression in mycelial growth to such a high extent comparable to that induced by the whole cotton dust mixture. This has been further emphasized by determining mycelial weights and their percentage decreases in comparison with controls (Fig. 5 and Table IV). These results draw the attention towards the necessity of studying the effect of pairs of cotton dust components.

(*iii*) *Effect of pairs of cotton dust components.*

The growth of either one of the three fungi, as expressed by lateral mycelial spread or by mycelial weights, has been studied in response to the following combinations of cotton dust components sulphur plus DDT (S + D), sulphur plus BHC (S + B) and DDT plus BHC (D + B). These different combinations, were added in the same concentrations present in 10,000 p.p.m. cotton concentration. With regard to the relative mycelial spread (Fig. 4), the three fungi behave more or less similarly in their growth responses towards any of the different combinations. The mixture of sulphur and DDT does not result in causing any appreciable change in mycelial lateral spread. On the other hand, the association of BHC with either sulphur or DDT results in inhibiting the mycelial growth in a more or less similar manner to that induced by cotton dust mixture as a whole. In case of *Sclerotium,* the addition of Gammexane to either sulphur or DDT is proved to be equally effective in causing complete inhibition of the fungal growth in quite a similar manner to that achieved by cotton dust mixture as a whole. On the other hand, in case of either *Fusarium* or *Botrytis,* the gammexane component is relatively more effective — in

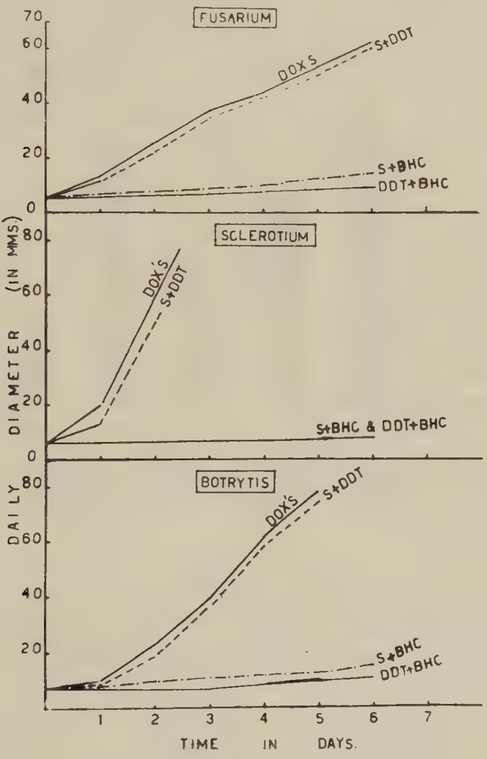

Fig. 4. Growth curves of the three fungi on medium containing different combinations of cotton dust components.

suppressing mycelial growth — when present in combination with DDT than when with sulphur.

These results have been further emphasized by comparing mycelial dry weights on different cotton dust treatments and determining the percentage decreases (Fig. 5 and Table III).

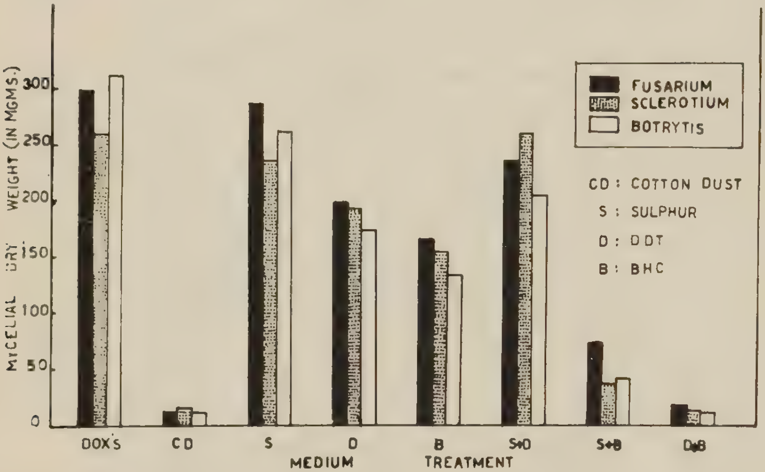

Fig. 5. Dry weights of the three fungi grown on Dox' liquid containing 1% of cotton dust either as a whole, as individual components or as combinations.

TABLE III.

Relative dry weights (W.R.) in milligrams — of either *Fusarium, Sclerotium* or *Botrytis* species — in response to Dox' liquid and to the same medium containing either cotton dust as a whole or its constituents singly or in combination, percentage decrease (P.E) in mycelial weight is also indicated in comparison with that on pure Dox' liquid.

Medium treatment	Experimental fungus					
	Fusarium		*Sclerotium*		*Botrytis*	
	(W.R)	(P.E)	(W.R)	(P.E.)	(W.R)	(P.E)
(Dox)	300	—	260	—	312	—
(Cotton dust)	12	96 %	15	94 %	12	96 %
(Sulphur)	288	4 %	238	8 %	263	16 %
(DDT)	200	33 %	195	25 %	176	44 %
(BHC)	168	44 %	156	40 %	135	57 %
(Sulphur + DDT)	238	21 %	260	(No change)	207	34 %
(S + BHC)	75	75 %	38	84 %	43	86 %
(DDT + BHC)	19	94 %	13	95 %	12	96 %

The results could be summarized as follows:

(1) With regard to the effect of individual insecticidal components of cotton dust, as manifested by relative percentage

decreases in fungal mycelial weights in comparison with the control, the most suppressive effect exerted by the Gammexane constituent, followed by DDT and the least by sulphur. The most responsive fungus — concerning mycelial suppression — is *Botrytis*, followed by *Fusarium* and the least is *Sclerotium*.

(2) The presence of sulphur, in combination with DDT, results either in complete elimination of the suppressive effect exerted by the latter constituent (i.e. *Sclerotium*) or in its reduction as shown by the lowering of the percentage decrease in mycelial weight (i.e. *Fusarium* and *Botrytis*).

(3) The combined effect of Gammexane with either sulphur or DDT is not of an additive nature but seems to be due to the resultant interaction between the two mixed components.

Thus, the actual inhibitory factor in cotton dust mixture — so far as the mycelial growth of the three experimental fungi is concerned is Gammexane constituent when mixed with either sulphur or DDT; its inhibitory effect is, on the whole, more pronounced in combination with DDT than with sulphur.

The germinative potentialities of either *Fusarium* or *Botrytis* conidia, in response to different treatments of cotton dust and its components could not be carried out owing to experimental difficulties resulted due to the relative insolubility of cotton dust and its constituents in water. The possible application of organic solvent for their dissolution would affect spore germination and lead to inconclusive results.

B. Effect of 2, 4-D

The growth responses of the three experimental fungi — expressed as mycelial lateral spread as well as dry weight — have been determined in relation to varying concentrations of the herbicide 2, 4-D (i.e. 100, 500, 2500 and 10,000 p.p.m.). In addition, the germinative potentialities of *Fusarium* microconidia and *Botrytis* conidia are tested in response to varying concentration of the soluble herbicide.

(*i*) *Mycelial growth*:

Growth curves of the three fungi on Dox' agar, either alone or containing varying concentrations of 2,4-D (Fig. 6), indicate gradual depression in mycelial lateral spread with increasing herbicidal concentration. The relative growth responses differ, however, according to the nature of the experimental fungus. On the whole, on Dox' agar containing 100 p.p.m. concentrations, the growth curve does not vary appreciably from that on pure Dox' agar. At higher experimental concentrations, *Botrytis* is found to be less tolerant than both *Fusarium* and *Sclerotium*. Thus, at 500 p.p.m. concentration, the depression in *Botrytis* mycelial lateral

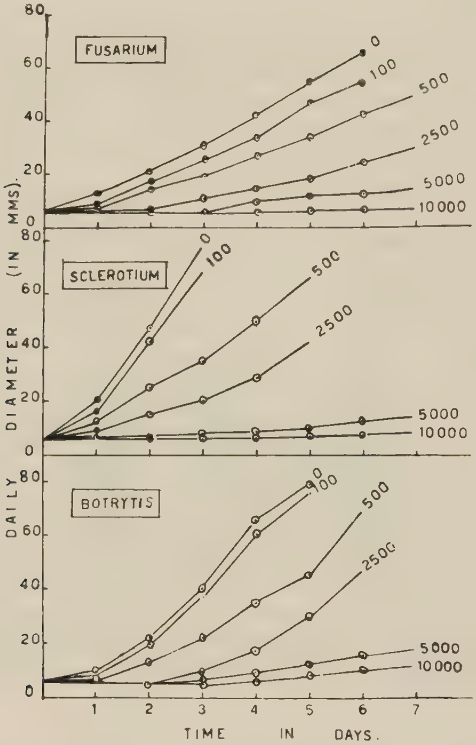

Fig. 6. Growth curves of the three fungi on Dox' agar containing different concentrations of 2,4-D.

spread becomes comparatively considerable in relation to that on the control while such depression is slight in case of *Fusarium* and *Sclerotium*. Similarly, complete inhibition of *Botrytis* mycelial growth occurs at 5000 p.p.m. concentration while such inhibition does not take place except at 10,000 p.p.m. 2,4-D in *Fusarium* and *Sclerotium*; at 5000 p.p.m. the mycelial lateral spreads of the two latter fungi were merely considerably suppressed. This observation emphazises the relative action of the herbicide towards the three experimental fungi.

The results of growth responses of the three fungi, as expressed by relative mycelial lateral spread, have been further confirmed by mycelial weight determinations on liquid media and by calculating percentage decreases in comparison with controls (Fig. 7 and Table IV). The results could be summarized as follows:

(1) As could be deduced from the relative percentage decreases, at both 100 p.p.m. and 500 p.p.m. 2,4-D concentrations, the most tolerant fungus is *Sclerotium*, followed by *Fusarium* and the least is *Botrytis*. At 500 p.p.m. concentration, the percentage decrease

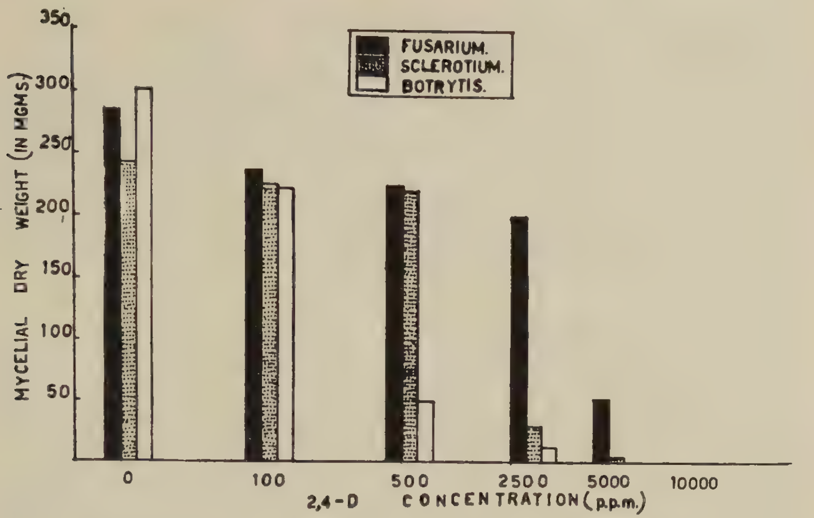

Fig. 7. Relative mycelial dry weights on Dox' liquid containing varying concentrations of 2, 4-D.

TABLE IV.

Relative mycelial dry weights (in mgms) — of either *Fusarium, Sclerotium* or *Botrytis* in response to Dox' liquid and to the same medium containing varying concentrations of 2,4—D; percentage decrease in mycelial weight is indicated in comparison with that on pure Dox' liquid.

Experimental fungus	*Mycelial dry weight (in mgms)*					*Percentage decrease*			
	0 (p.p.m)	100 (p.p.m)	500 (p.p.m)	2500 (p.p.m)	5000 (p.p.m)	100 (p.p.m)	500 (p.p.m)	2500 (p.p.m)	5000 (p.p.m)
Fusarium	286	237	225	200	52	17 %	27 %	30 %	82 %
Sclerotium	242	225	220	30	5	7 %	9 %	88 %	98 %
Botrytis	300	220	50	13	No growth	27 %	83 %	96 %	—

in *Botrytis* mycelial weight is considerably high and is almost comparable to that attained by *Fusarium* at 5000 p.p.m. concentration.

(2) At 2500 p.p.m., the relative tolerance is reversed as far as *Fusarium* and *Sclerotium* are concerned; *Fusarium* becomes highly tolerant in comparison with *Sclerotium.* On the other hand, *Botrytis* mycelial growth becomes very considerably suppressed.

(3) At 5000 p.p.m. concentration, *Botrytis* mycelial growth becomes completely inhibited. On the other hand, *Sclerotium* mycelial growth is considerably suppressed to such an extent which nearly approaches an inhibition while *Fusarium* growth is merely greatly retarded.

(4) At 10,000 p.p.m. the mycelial growth of either *Fusarium* or *Sclerotium* is completely inhibited.

The complete failure of the three fungi to manifest any sign of mycelial growth at the highest experimental 2,4-D concentration, even when left incubated for one month or more, direct the attention towards finding out whether the herbicidal action is fungistatic or fungicidal at such high concentration. After reinoculating each of the arrested fungi on pure Dox' medium, no sign of growth could be detected indicating the fungicidal nature of 10,000 p.p.m. 2,4-D concentration on the three fungi.

(*ii*) *Spore germination*:

The germinative potentialities of the two sporulated fungi, namely *Fusarium* and *Botrytis*, were tested in response to 100, 500, 2500 and 10,000 p.p.m. 2,4-D solution; these were compared with the germinative capacities on pure water as control. The technique adopted was the "hanging drop method" using Van Tieghem cells. Incubation was carried out at a temperature about 20° C. The germinative potentialities have been expressed by the following criteria.

(1) Latent period of germination.

(2) Rate of elongation of germ tubes, every four-hours interval, for 24 hours.

(3) Final percentage germination after 24 hours.

A comparison of the latent period of germination on pure water, of *Fusarium* microconidia, on one hand and of *Botrytis* conidia, on the other hand, shows that the latter fungus has a higher latent period (Fig. 8). At 100 p.p.m. concentration, the latent periods of germination — for the conidia of the two fungi — are similar to those on pure water. On the other hand, at concentrations above 100 p.p.m., the latent periods of germination become successively prolonged with increasing concentration of 2,4-D; these of *Botrytis* conidia are more prolonged — in comparison with control — than in case of *Fusarium* microconidia. At 10,000 p.p.m. concentration, *Botrytis* conidia cease completely to show any sign of germination even after 48 hours incubation while germination of *Fusarium* microconidia is considerably retarded.

The relative tolerance of *Fusarium* microconidia and the high sensitiveness of *Botrytis* conidia, in their germinative responses to increasing concentrations of 2,4-D, have been also demonstrated by comparing the germination percentage after 24 hours. In case of *Fusarium*, the germination percentages remain more or less similar on pure water as well as aqueous solutions containing comparatively lower experimental 2,4-D concentrations up to 2500 p.p.m. At 5000 p.p.m. concentration, *Fusarium* conidial germination percentage becomes considerably decreased and a very low value is attained at 10,000 p.p.m. On the other hand,

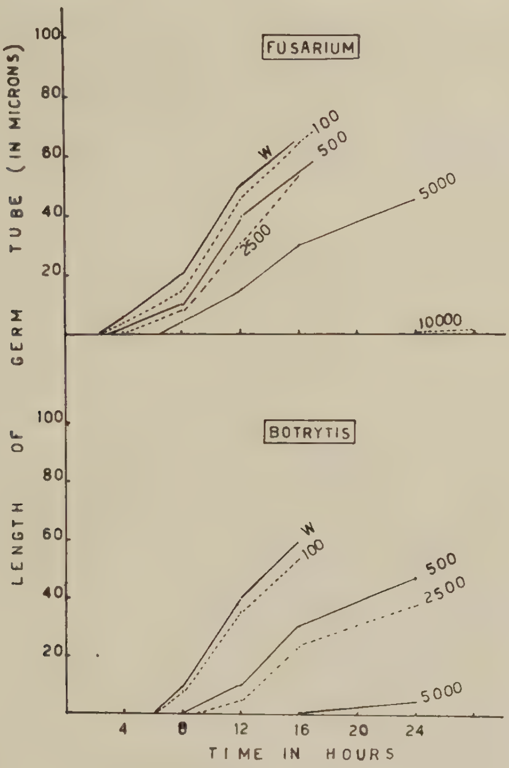

Fig. 8. Germinative capacities of fungal conidia on pure water (W) and different concentrations of 2, 4-D.

in case of *Botrytis* conidial germination, percentage become considerably decreased — in comparison with that on pure water-at a comparatively low 2,4-D concentration (i.e. 500 p.p.m.); the germination percentage continues to be successively considerably decreased — with increasing concentration above 500 p.p.m. — until *Botrytis* conidia cease to germinate at 10,000 p.p.m. 2,4-D concentration.

IV. General Discussion

Owing to the difference in the relative persistency of cotton-dust insecticidal components, it has been found necessary to eliminate their effects — on fungal vigour — when they are present as individuals or as combination of pairs. Previous literature indicates that these insecticidal components viz., sulphur, DDT and BHC were studied as individuals with regard to their effects on micro-organisms (Wilson & Choudhri (1946 and 1948), McFarland 1946). The importance of the present work lies in the fact that, in addition to the evaluation of individual effects of cotton dust insecticidal components, studies have been further extended to include the combined effects of pairs of the components as well as their resultant effect in cotton dust as a whole.

High concentrations of cotton dust have been used in the attempt to elucidate the following points:

(1) High local concentrations due to uneven dusting with cotton dust.

(2) High concentration as a result of the accumulation effect of the insecticide, due to successive dusting in the future.

(3) The evaluation of cotton dust concentration, which would be partially or completely inhibitory to fungal growth.

With regard to the effect of cotton dust on fungal growth, it was found that up to 2500 p.p.m. concentration fungal mycelial growth was found to decrease successively with increasing cotton dust concentrations, *Botrytis* is found to be comparatively more sensitive followed by *Sclerotium* and the least is *Fusarium*. The mycelial growth of the three fungi is found to be more or less equally suppressed at 5000 p.p.m. and do not become nearly ceased except at 10,000 p.p.m. The actual inhibitory component in cotton dust — responsible for depressing fungal vigours — is similarly found to be due to the Gammexane component when mixed with either sulphur or DDT, its inhibitory effect is on the whole, more pronounced in combination with DDT than with sulphur.

2,4-D is similar to cotton dust in the fact that fungi are tolerant to its high concentrations. *Botrytis* is found to be more sensitive to the herbicide inhibitory action than either *Fusarium* or *Sclerotium*. Such relative potentiality, towards microorganisms, have been also recorded by GARBER and co-workers (1951). A general comparison of the mycelial growth responses of the three experimental fungi, to comparable increasing concentrations of either cotton dust or 2,4-D has yielded the following results:

(1) At 100 p.p.m. *Botrytis* is found to be the most sensitive fungus in response to either cotton dust or 2,4-D. This holds also true at 500 p.p.m. concentrations; the percentage decreases are however, considerably greater on 2,4-D than on cotton dust.

(2) At 5000 p.p.m. concentration of 2,4-D, *Botrytis* mycelial growth has been found to be completely ceased. On the other hand, no cessation of *Botrytis* mycelial growth takes place on a similar cotton dust concentration.

(3) At 10,000 p.p.m. 2,4-D concentration, mycelial growth of either *Fusarium* or *Sclerotium* completely ceases while they still show very feeble mycelial development on a similar concentration of cotton dust.

On the whole, fungi are comparatively more tolerant to the action of cotton dust than that of 2,4-D. The selective action of 2,4-D towards *Botrytis* has been further confirmed by the germination potentialities of its conidia in response to the herbicide. In case of the two sporulated fungi, namely *Botrytis* and *Fusarium*, the concentrations of 2,4-D necessary for complete cessation of mycelial growth are comparatively lower than those required to achieve complete inhibition of conidial germination. 10,000 p.p.m. 2, 4-D concentration was found to be completely fungicidal for mycelial growth. On the other hand, although a few conidia germinate at much higher concentration, yet the germ tubes become gradually toxicated and further development would soon stop. The toxicity of 2,4-D derivatives, to either mycelial growth or spore germination, has been similarly recorded by other investigators (STEVENSON & MITCHELL, 1945, LEWIS & HAMNER, 1946, GUISCAFRE-ARRILLAGA, 1948, BEVER & SLIFE, 1948, WEI & LING, 1949, IBRAHIM, 1951, PETURSON, 1951, GARBER and coworkers, 1951 and HSIA & CHRISTENSEN 1951).

SUMMARY

The present work aims at the elucidation of the effect of either cotton dust and its components or 2,4-D on the relative vigours of the three pathogenic fungi namely, *Fusarium solani, Sclerotium bataticola* and *Botrytis fabae. Botrytis* mycelial growth was found to be comparatively more sensitive to cotton dust than *Fusarium* and *Sclerotium.* The elucidation of the effect of individual cotton dust components has shown that Gammexane is the most effective inhibitory component while sulphur and DDT caused slight inhibition. This inhibitory effect of Gammexane is more pronounced when mixed with DDT than with sulphur. 500 p.p.m. 2,4-D concentration inhibited *Botrytis* growth more than *Fusarium* or *Sclerotium,* 1 % 2,4-D concentration was proved to be fungicidal. 2,4-D suppressed the germinative potentialities of *Fusarium* and *Botrytis* conidia.

ZUSAMMENFASSUNG

Die jetzige Arbeit trachtet nach der Aufklärung der Wirkung des Baumwollstaubes und seiner Bestandteile, und des 2,4-D auf die relative Widerstandsfähigkeit von drei pathogen Pilzen, nämlich, *Fusarium solani, Sclerotium bataticola* und *Botrytis fabae.* Mycelium-Wachstum von *Botrytis* war vergleichsweise empfindlicher gegen Baumwollstaub als von *Fusarium* und *Sclerotium.* Die Aufklärung der Wirkung der individuellen Komponenten des Baumwollstaubes zeigte, daß Gammexane der wirksamste, verzögernde Bestandteil ist, während Sulfur und DDT nur eine geringfügige Verzögerung verursachten. Die verzögernde Wirkung von Gammexane ist ausgesprochener, wenn es mit DDT als mit Sulfur gemischt ist. Die Konzentration von fünfhundert Teilen in 1 Million von 2,4-D unterdrückt das Wachstum von *Botrytis* mehr als das von *Fusarium* oder *Sclerotium,* die Konzentration von 1 % des 2,4-D war pilztötend. 2,4-D unterdrückte die Keimungsmöglichkeit von *Fusarium* und *Botrytis* Conidia.

References

BEVER, W. M. & SLIFE, F. W. (1948) The effect of 2,4-D. in culture medium on the growth of three pathogenic fungi; Phytopath., **38,** 1038.

FLEMING, W. E. (1950) Persistence effect of DDT on Japanese beetle larvae in New Jersy soils; J. econ. Entom., **43,** 87—89.

GARBER, H. H., SCHAAL, L. A. & FULTS, JESS L. (1951) The selective action of penta chlorophenoxyacetic acid against *Streptomyces scabies* (Thaxt.) Waksman & Henrici, in culture media: Phytopath., **41,** 991—996.

GUISCAFRE-ARRILLAGA, J. (1948) Sensitivity of *Penicillium digitatum* to 2,4—Dichlorophenoxyacetic acid; Rev. appl. Mycol., **27,** 471.

HSIA, Y. T. & CHRISTENSEN, J. J. (1951) Effect of 2,4-D on seedling blight of wheat caused by *Helminthosporium sativum*; Phytopath. **41,** 1011—1020.

IBRAHIM, I. A. (1951) Effect of 2,4-D on stem rust development in oats; Phytopath., **41,** 951—953.

LEWIS, R. W. & HAMMER, C. L. (1946) Effect of 2,4-D on some microorganisms: Mich. Agr. Expt. Sta. Quart. Bul., **29,** 112—114.

MCFARLAND, W. H. (1946) The fungicidal properties of DDT; Rev. appl. Mycol., **25,** 589.

PETURSON, B. (1951) Effect of growth promoting substances on the germination of uredospores of crown rust; Phytopath., **41,** 1039—1040.

SMITH, M. S. (1948) Persistence of D.D.T. and benzene hexachloride in soils; Ann. appl. Biol., **35,** 494—504.

STEVEN, E. C. & MITCHELL, J. W. (1945) Bacteriostatic and bacteriocidal properties of 2,4-D; Science, **101,** 642.

WEI, C. T. & LING, K. C. (1949) The effect of 2,4-D on spore germination of fungi; Rev. appl. Mycol., **28,** 70.

WILSON, J. K. & CHOUDHRI, R. S. (1946) Effects of DDT on certain microbiological processes in the soil; J. econ. Entom. **39,** 537.

WILSON, and K. & CHOUDHRI, R. S. (1948) The effect of benzene hexachloride on soil organisms; J. agric. Res. **77,** 25.

A REVIEW OF FUNGUS INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

by

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(30.III.1960)

No longer is the premise valid that fungus infections in man are considered as harmless infections. Until recently there were a few and certainly inaccurate vital statistics on fatalities resulting from fungus infections because very rarely were these recorded as causes for death. In 1951 the United States Vital Statistics reported that 338 had succumbed to systemic fungus diseases. We are certainly not aware of the great number who are disabled with residua and sequelae of these very important causes for illness.

Nickerson has stated that "fungus infections are probably the most widely distributed and most numerous types of infections" (1). It is only within the past ten years that there has been a tremendous resurgence of interest in the identity and management of these diseases and now numerous medications are appearing regularly in the armamentarium of the physician to help combat these infections. Prior to the last decade there was little, if any, specific management known. As recent as 1956, L. Boshes, et al. noted that, "It is apparent that the clinician remains powerless in most instances in the management of fungus infections" (2). However, the author no longer feels that this premise is correct for, at the present time, new and truly effective agents have appeared with most promising results in those fungus infections which heretofore had been most rigidly resistant to treatment. At the present time, I might point out that there are only four so-called "deep fungus infections" which have specific management, namely, coccidioidomycosis, nocardiosis, blastomycosis and actinomycosis. In the other fungus infections that I will discuss there is, to the present, no specific therapy. Some of the newer anti-fungal agents have resulted in clinical and bacteriological arrest. Whether these are only periods of remission or even recoveries remains to be seen for several of these illnesses are characterized by exacerbations and remissions. In addition to specific medications, the usual management to include general therapy, bedrest, adequate diet, transfusion, vaccines, vitamins, dyes, steroids and even reassurance are still being employed. The following are the more important and most commonly encountered fungus infections. Their activity within the central nervous system will be considered.

CRYPTOCOCCOSIS

The etiologic agent is the *Cryptococcus neoformans*, the only yeast known to infect and kill animals and man. The disease is also known as European blastomycosis and torulosis and was first described in humans in 1894. It may occur in any area of the world and effects all ages and both sexes. The organism, in all likelihood, is disseminated in the soil where it leads a benign existence. Pasteurization may kill it in course.

Cryptococcus neoformans may be recognized in spinal fluid directly by an India ink preparation (3) (Figure 1). It can be grown quite easily on Sabouraud's glucose agar at room temperature. The yeast-like cells may be seen on direct examination of the spinal fluid and are ovoid or spherical single-budding, thick-walled, 5—20 μ in diameter, with wide gelatinous and refractile capsules staining gram positive (Figure 2).

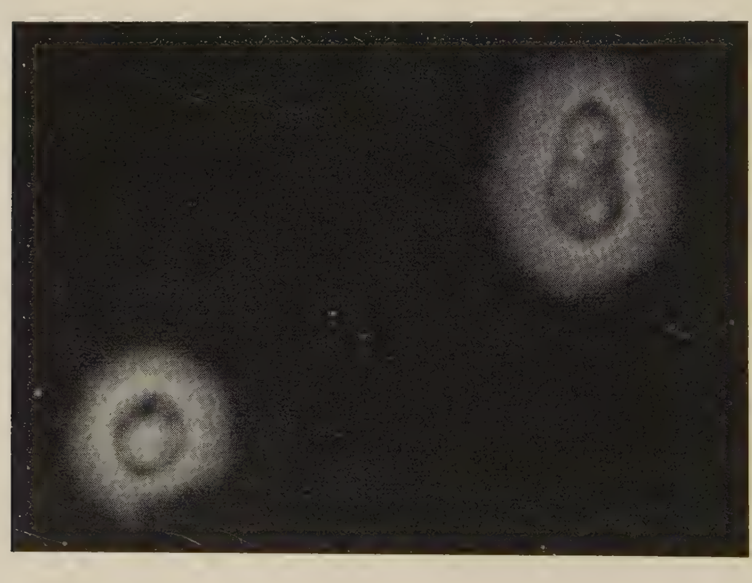

Figure 1
India ink preparation of urine showing budding *C. neoformans* by direct smear X 500

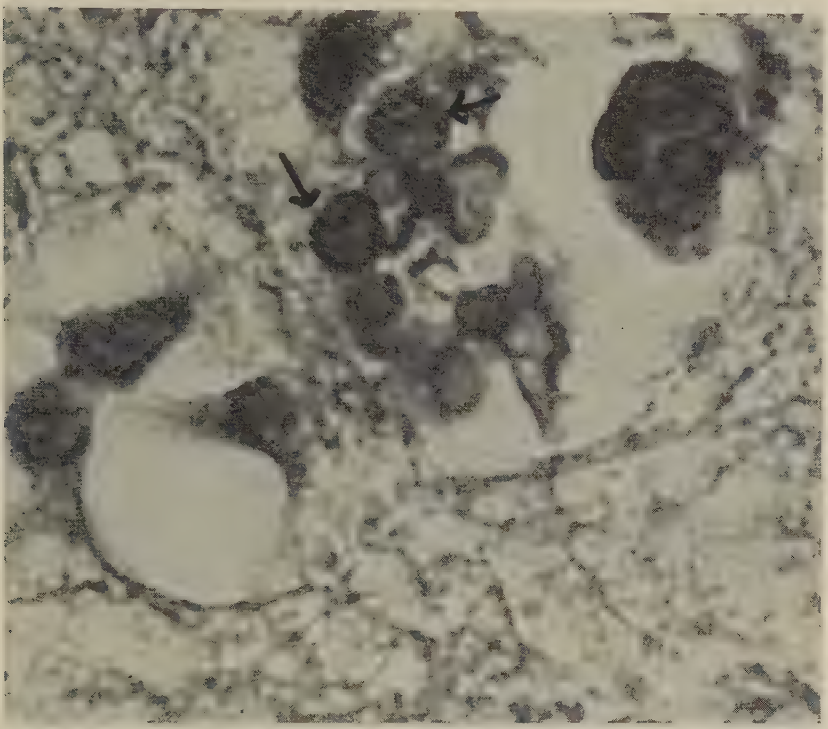

Figure 2
Section through histolytic lesion in basal ganglia. Organism easily noted. PAS X 900
Arrow points to organism.

Pathology:

Mosberg & Arnold (4) have described the rather complete pathological picture of this disease complex as it affects the central nervous system. Recently L. Boshes, et al. reported a series of these cases each with a different yet frequently reported type of onset (2). Collins and his group (5) have emphasized that cryptococcosis may be seen co-existing in conditions of malignant lymphomas whereas others have pointed out that this illness may co-exist in endocarditis, diabetes, pyelonephritis and may be seen in conjunction with other fungus infections (6). Grossly the brain reveals granulomata and cysts in the cortex with honeycomb areas of histolysis in both basal ganglia. These cyst-like areas contain colloid material (Figure 3). Microscopically, sections made through these areas of histolysis and especially along the course of blood vessels reveal the so-called "scap-suds" appearance (Figure 4). A meningitis and/or a meningo-encephalitis may be present (Figures 5 and 6).

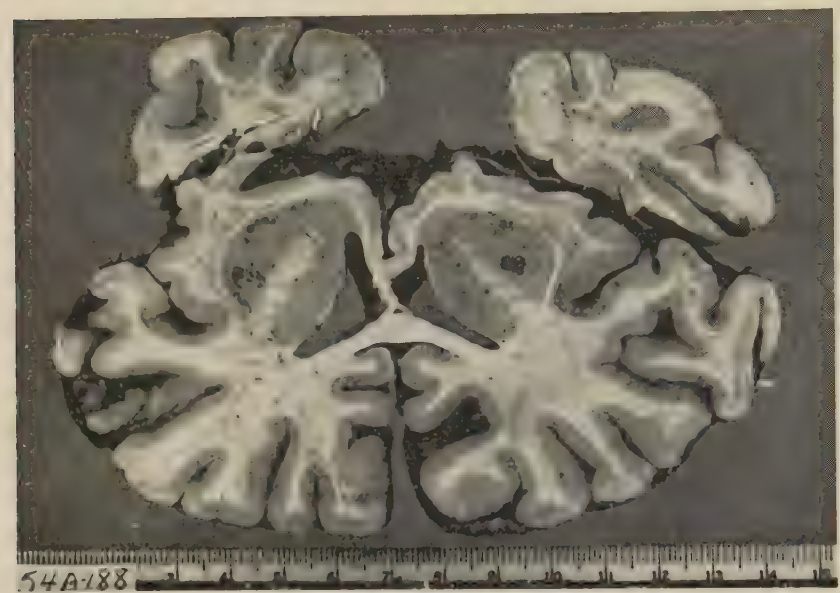

Figure 3
Coronal section of brain showing lesions in the basal ganglia.

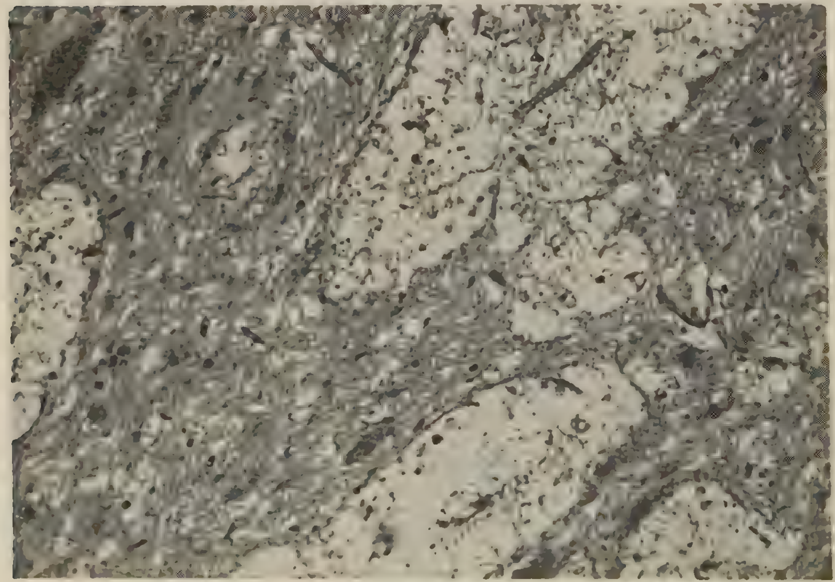

Figure 4
Section through area of histolysis in basal ganglia typical soap-suds appearance.
H& E × 75

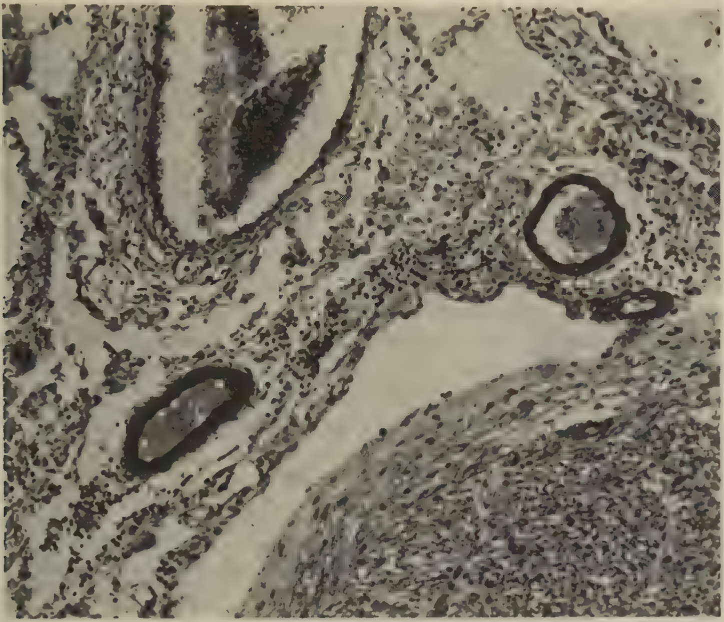

Figure 5
Meninges of medulla revealing a meningitis. PAS × 100
In higher magnification the Cryptococci could be recognized easily.

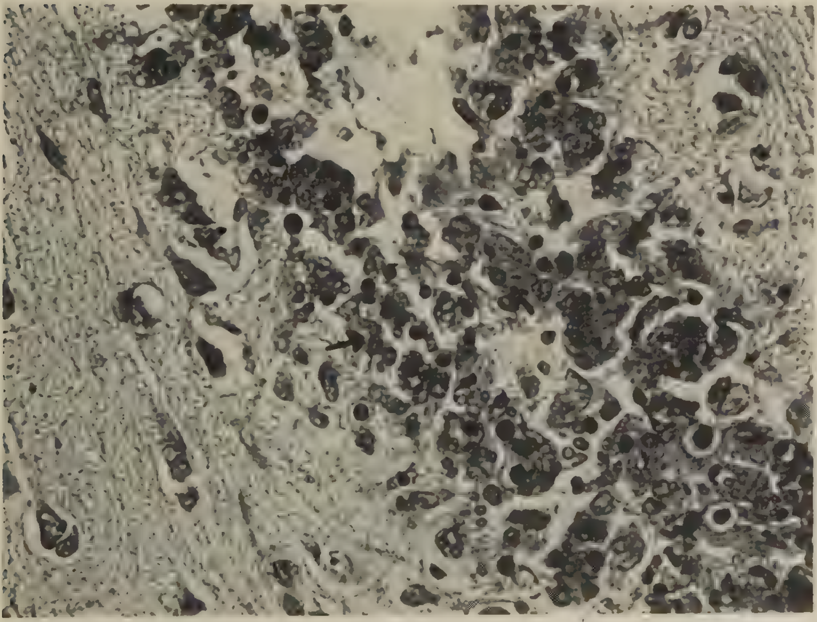

Figure 6
Biopsy of leptomeninges. Meningitis and organisms easily seen. Arrows point to organisms. PAS × 500

Symptomatology:

Not too uncommonly have appeared reports of cases of cryptococcosis which have been incorrectly diagnosed as some form of chronic meningitis such as tuberculous or choriomeningitic. All of these may reveal pleocytoses and an elevated protein with decreased glucose and chloride content in the spinal fluid. However, tuberculosis should not be accepted as a final diagnosis until the tubercle bacillus is demonstrated or at least until all other fungal diseases are excluded, especially cryptococcosis. Not infrequently is reported a case of cryptococcosis with marked increase in the intracranial pressure to suggest the diagnosis of a brain tumor. Actually, the entire symptom complex may be the result of a large granuloma composed almost entirely of organisms. The usual history is that of an individual in apparent good health until a relatively mild cranial injury such as a bump or blow to the head will cause the picture of increased intracranial pressure. Spinal fluid will be under increased pressure and may be clear, turbid, or even xanthochromic, containing up to several hundred cells per cubic millimeter, most of these lymphocytes. Both sugar and chloride levels are reduced, as in meningitis, while the protein is elevated. Martin & Padberg and Wilson & Duryea have reported cases of cryptococcosis resembling the picture of subdural hematoma whereas Ley et al. described a torulogranuloma which was diagnosed as a cervical cord tumor in an eight-year-old girl. (8), (9, (10).

The diagnosis of cryptococcosis, then, is not too difficult to make providing one entertains the possibility of this causing an acute or a chronic meningo-encephalitis. Also, where there are cases of long existing headache, one must consider fungus infection and at times even foremost.

Treatment:

Until only a few years ago, there were numerous managements employed in cryptococcosis but most of there were to no avail. Fatalities ordinarily occurred during the fulminating stage of the disease, or some patients would live for many years in a condition of chronic invalidism. A few more fortunate patients were reported in remissive periods as long as 20 years. Actidione, a new antifungal cyclohexamide, appeared on the scene some years ago and was quickly accepted as a specific drug for the treatment of cryptococcosis but very rapidly it was evident that there were too many complications resulting. L. Boshes et al. (2) felt that the drug even precipitated the death of one of the patients in their group while Wilson & Duryea discontinued this medication in their patients when they would develop lethargy, ataxia and slurred speech, even though there was a marked improvement in the spinal fluid which became sterile and a clinical arrest in the symptom picture (9).

Nystatin has been found to be ineffective. PROCKNOW & LOOSLI reported several cases of improvement with 2-hydroxystilbamadine (11). Mycostatin, another of the newer antibiotic preparations, has shown some promise in the management. Cortisone and ACTH have been used but to the present little, or no, good effect has been observed. Recently, GOLD and his group isolated a streptomycete culture possessing antifungal activity from the soil in the Orinoco Region of Venezuela (12). The investigators attributed this specific activity to two related antibiotics, namely, Amphotericin A and Amphotericin B, which later were isolated in crystalline form by VANDEPUTTE and his associates (13). In the recent past, several reports have appeared in the literature of apparent success achieved in the treatment of cryptococcosis with Amphotericin B. BIDDLE & KOENIG reported two such cases with apparent bacteriologic and clinical arrest(14).

COCCIDIOIDOMYCOSIS (SAN JOAQUIN FEVER, DESERT RHEUMATISM)

This disease is quite endemic in Arizona, New Mexico, southwest Texas and California but may be found even elsewhere in the United States, Mexico, Venezuela, Paraguay and Argentina. Actually, it was recognized as early as 1892 but it continued to mount in importance until in World War II when there were 6000 cases reported in American troops stationed in the southwest area of the United States. Of this group, 50 died. All ages and both sexes are equally involved and dark-skinned races seem to be more prone to develop the granulomatous form of the disease. It has been estimated that almost 15 per cent of newly arrived residents in coccidioides areas contract the infection within the first year with or without any apparent symptoms. This disease has even been reported in animals and, oddly, has been found only in the boxer dog to date.

The organism is usually isolated from spinal fluid and may be identified in the tissues with P.A.S. stain (periodic-acid-Schiff reagent) or the Hotchkiss-McManus technique(15). This is a thick-walled structure, 20—80 μ in diameter, filled with numerous 2—5 μ endospheres and is non-budding (Figure 7). At the present time, the Gömöri methenamine silver stain, a new technique, is employed quite frequently to demonstrate the fungus.

When coccidioidomycosis invades the nervous system, this is usually the result of a dissemination of the infection from a primary site and most of the time this is in the lung. Practically every case to date has proven fatal but there have been apparent recoveries reported(16). FORBUS has reported that 10 per cent of his patient group developed central nervous system involvement well confined to the meninges and SCHLUMBERGER also noted an incidence of 13 cases of basilar meningitis in 23 cases of disseminated coccidioidomycosis (17, 18), ABBOTT & CUTLER, on the other hand reported an incident of only 25 per cent of their cases with central nervous

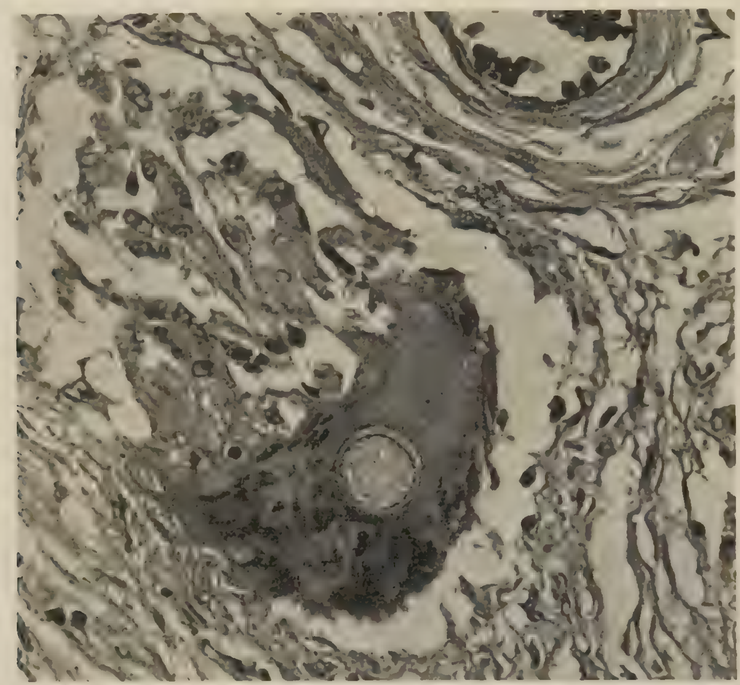

Figure 7
Spinal meninges. Giant cell containing *C. immitis*. H × 8 × 500

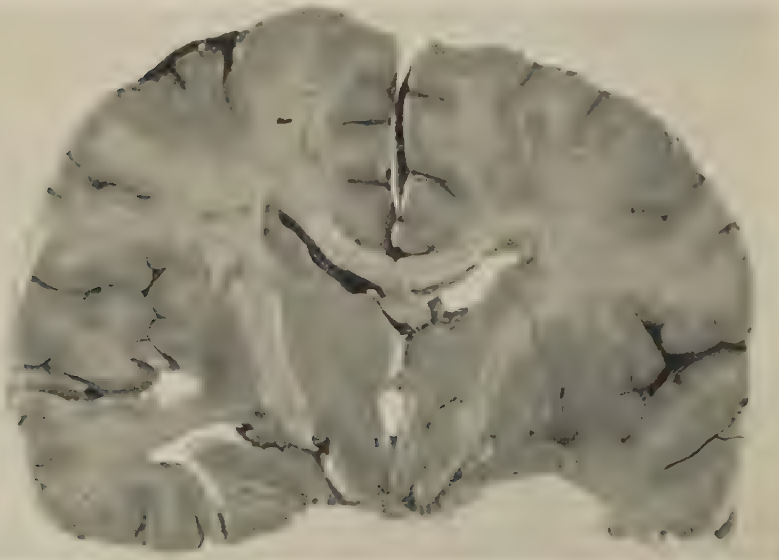

Figure 8
Gross brain section showing basilar meningitis.

system involvement(19). It should be remembered that the primary site of entry may escape detection at autopsy so that not always is central nervous system involvement apparent.

Pathology:

Characteristically there is seen a thick, fibrous, meningeal exudate around the base of the brain suggesting a clinical picture of a meningeal encephalitis or even a neoplasm(20). At times the granuloma may even resemble a spinal cord tumor(21) (Figures(8), (9), (10), (11).

The cerebral spinal fluid picture is praxtically that of a tuberculous meningitis and may be clear, turbid, or xanthochromic. The cells may range from several hundred to 2600 per cubic millimeter. Sugar and chloride content is usually diminished while protein is elevated. In some cases the organism may be isolated from fluid or may be identified otherwise in tissues with the stains already mentioned.

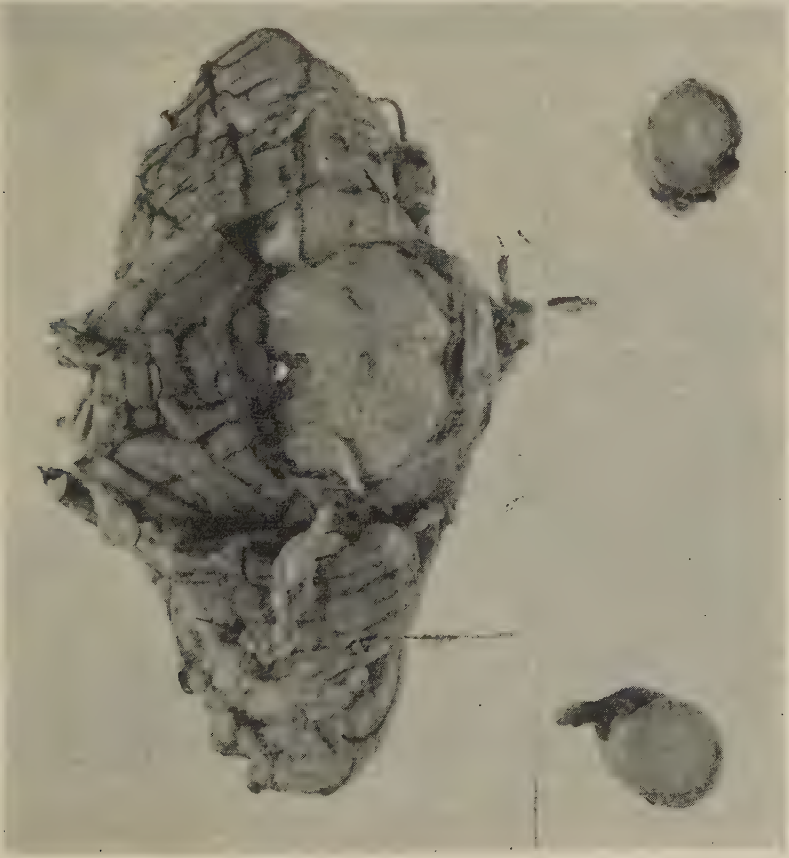

Figure 9
Gross section of brain stem and spinal cord showing thick lepto-meningitis.

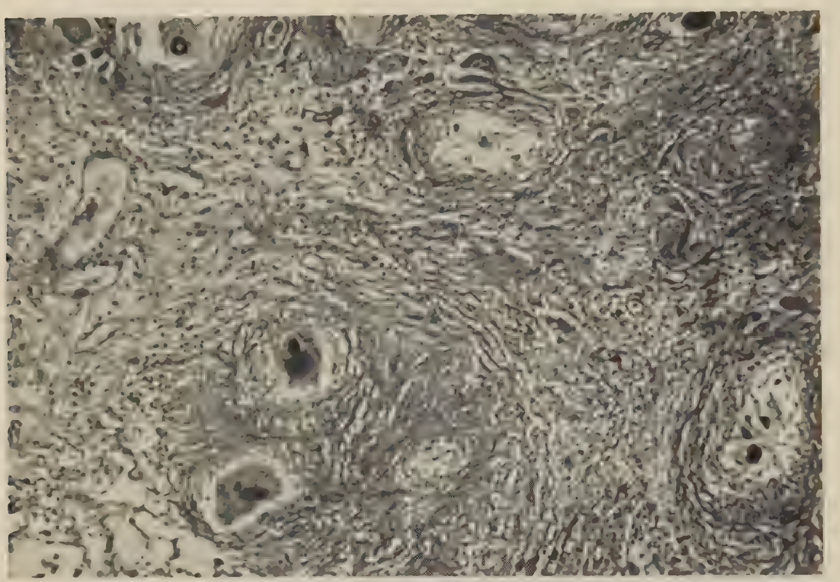

Figure 10
Basilar meninges. Tubercle like structures containing large giant cells in which are seen *C. immitis*. PAS × 125

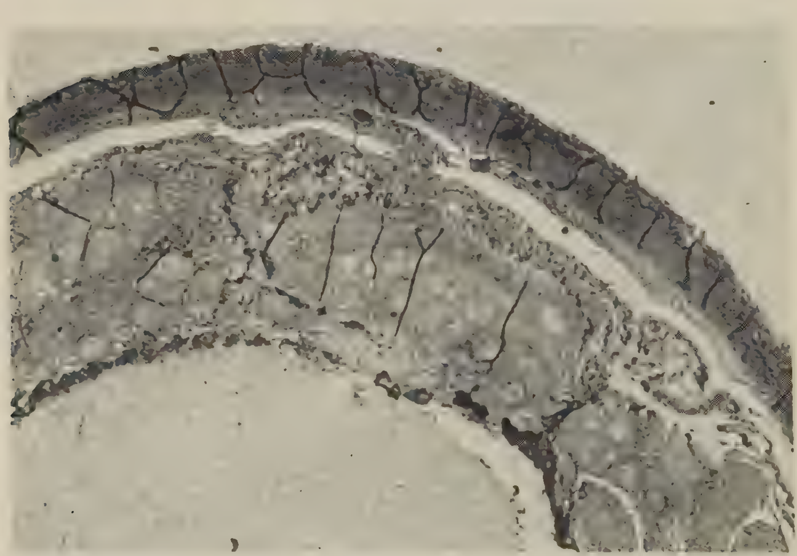

Figure 11
Spinal cord granulomatous meningitis with softening. Van Gusen × 25

Symptomatology:

The clinical diagnosis of coccidioidomycosis is dependent upon a healthy suspicion and awareness in individuals from endemic areas in whom there has been pulmonary involvement which has not cleared up within a reasonable length ot time under the usual regime. Quite often no organism may be identified from the fluid, either by smear or culture but only by complement fixation and precipitin tests on the serum and on the spinal fluid. Until recently, death occurred within 4 to 5 years although several cases of survival for 10 years without any specific treatment are on record(22).

Treatment:

The most effective drug in the management of this illness at present is Amphotericin B. This is considered fungicidal if administered early and fungostatic if given later. In the case reported by KLAPPER, SMITH & CONANT, their patient was involved over a period of 10 years and tolerated the drug rather well for a period of some 14 months. As adjunctive management, Mycostatin was used. Amphotericin B has been administered intrathecally in some instances(23). Others have used aminostilbamadine with only measured success(24). L. BOSHES and his group were quite disappointed in their results with Actidione and JENKINS & POSTELWAITE reported similar results in their two cases (2, 20). In addition, arsphenamine crystals, thymol, related oils, and gentian violet are still being used. Most of the newer antibiotics have failed to be of benefit in the treatment of disseminated coccidioidomycosis in the central nervous system.

ACTINOMICOSIS AND NOCARDIOSIS

In the order of Actinomycetales are included *Actinomyces bovis* and *Nocarda asteroides*.These lie in an intermediate position between the true bacteria and other fungi. Actinomycosis of the central nervous system is not too frequently seen but when an infection does occur, it usually results in a meningitis with or withour a brain abscess. Actinomycosis is also known as "lumpy jaw". This specific fungus has a world-wide distribution and range and seems more common in rural than in urban areas. It occurs usually in the fourth decade of life and seems to single out man in preference to woman. The common types of strains are believed to be *Actinomyces israeli* for the human and *Actinomyces bovis* for the bovine species.

Infection of the central nervous system generally follows a primary infection elsewhere in the body. The spread is through the meninges by direct extension from a primary focus in the soft tissues or the bony cavities of the head. Generally the jaw is involved but any other portion of the face, mouth, throat, ear of nasal sinuses may be involved. The infection may enter the cranium via the foramina at the base of the skull. An infection in the lungs, bronchial glands, gastro-intestinal tract or even the skin may be the source by which the blood stream may carry the organism to the central nervous system. Once there is a generalized septicemia, other secondary lesions may result elsewhere in the body.

Pathology:

There may be a varied form of reaction. Meningitis is most common. The inflammatory exudate in the subarachonid space is thick and yellowish and is composed chiefly of leucocytes, fibroblasts and fungi. These are seen in granules as lobulated bodies composed of delicate branching intertwined filaments 1 m in diameter. These club-shaped structures are seen in optical sections around the periphery giving the picture of a typical granule with clubs - hence the name "ray fungus". They are gram postive characteristic "sulfur granules", are present in abscesses and draining sinuses.

A brain abscess which may exist with or without a meningitis is another pathologic feature.

Symptomatology:

This depends upon the type and location of the pathological involvement. Focal signs or symptoms generally seen with a brain abscess may produce hemiparesis with the usual associated reflex changes, convulsions, and visual disturbances. Other signs include headache, dizziness, vomiting, papilledema, meningeal signs, delirium, and finally, coma. The fever rarely exceeds 102° F.

Laboratory findings:

The white count may vary between 15,000 and 25,000. The cerebrospinal fluid is usually purulent and bright yellow, and occasionally may be so thick that it cannot flow through the ordinary spinal puncture needle. Cells may number up to several hundred per cubic millimeter and are usually polymorphs. Chlorides and glucose are usually decreased and protein is generally increased.

Treatment:

Heretofore, practically every case of actinomycosis with meningitis succumbed. Now, the combination of penicillin and sulfonamide therapy, or penicillin and streptomycin combinations, have helped many cases (11). Some of the newer drugs have been employed, to include tetracycline, erythromycin, and chloramphenicol, but penicillin in massive doses and even surgical drainage may result in successes up to 50 per cent (25, 26, 27).

NOCARDIOSIS

Nocardia asteroides (EPPINGER, 1894) is probably the most common pathogenic species seen in this country. This is a chronic granulomatous involvement which may affect all viscera including the brain and spinal cord. The organism simulates an actinomycosis but does not have the sulfur granules and the usual yellow exudate.

Sinus formations are rarely seen and this disease may resemble a tuberculosis. The organisms are generally gram positive or partially acid fast delicate intertwining branched hyphae that often break up into bacillary forms.

Treatment:

The treatment of choice includes sulfonamides, such as sulfadiazine and sulfamerazine (28) or sulfadiazine and streptomycin. Gantricin has been used quite effectively. RANKIN & JAVID (29) at the University of Wisconsin Medical School reported a case of nocardiosis of the central nervous system treated successfully with surgical excision. They also used chemotherapy and antibiotic therapy. RUNYAN believes that antibiotics should be substituted for the sulfonamides (30).

BLASTOMYCOSIS (GILCHRIST'S DISEASE, 1894)

NORTH AMERICAN TYPE

This is a mold disease which has been reported as being present in various areas of North America but chiefly in the central state area of the United States. It is generally seen in individuals engaged in farming and usually is present in men between the third to fifth decades of life. It is characterized by single and multiple granulomatous lesions of the skin which are usually painless, and with multiple granulomatous lesions in the internal organs. In the systemic form, the organism may have widespread dissemination following through the respiratory system. As a result, metastatic lesions may occur anywhere throughout the body, the pathway being via the bloodstream. Infections of the brain and meninges may be preceded by an involvement of the bone or soft tissues adjacent to central nervous system structure with subsequent direct spread to the nearby nervous structures.

Pathology:

Grossly, pathological changes certainly resemble those seen in tuberculosis. There are miliary abscesses containing the blastomycetes, multinucleated giant cells, plasma cells, round cells and even a great deal of necrotic material. The tendency to abscess formation is quite high.

Cerebrospinal fluid may be seen clear or turbid and the cell count rarely exceeds 500 per cubic millimeter. Glucose and chloride content is diminished whereas protein is elevated. The organism, the *Blastomyces dermatitidis,* is a single or budding spherical cell, 8—15 μ in diameter with thick refractile wall and there are no mycelia present.

Symptomatology:

Skin lesions are usually painless but symptoms are generally chronic. A chronic meningitis is generally seen as this part of the nervous system is concerned and this may last weeks, months or even longer. Occasionally, cranial nerve palsies are seen with focal signs. Coma finally appears and ordinarily the outcome is fatal. Diagnosis is made on the discovery of other systemic involvement of disease, by tissue biopsy and on the growth of the organism on a suitable culture medium. There is no complement fixation available for this illness.

Treatment:

The accepted treatment, which is almost specific in many cases, is 2-hydroxy-stilbamadine (31). Potassium iodide, used as long ago as 1894 by GILCHRIST, is still acceptable as a reliable form of management and may be curative in many cases. Vaccine and X-ray therapy are still being used as adjunctive, if not curative, measures. Mycostatin has been effective in many cases. The Amphotericin B has proven quite successful in resistant cases.

SOUTH AMERICAN TYPE

This is also a chronic granulomatous disease of the skin, mucous membranes, lymph nodes and internal organs. The offending organism is the *Blastomyces brasiliensis*, and is distinguished from *B. dermatitidis* or Gilchrist type by the multiple budding, yeast-like forms which are large, spherical and thick-walled. The clinical picture varies not too far from the North American form but more often resembles tuberculosis. There are numerous physical manifestations, including symptomatology due to invasion into the central nervous system.

Treatment:

The treatment is similar to that of the North American type.

ASPERGILLOSIS

This disease process is caused by a certain specis of Aspergillaceae and is characterized by the presence of inflammatory granulomatous lesions in eye, orbit, external ear, nasal sinuses, lungs, skin, bones. The meninges are involved not too frequently. Aspergilli always have been common and troublesome fungi contaminants in laboratories and hence a clearcut diagnosis is not too easy to make when cultures are in process.

There have been but few cases of aspergillosis recorded since the first report by MONIZ & LEFF(32) of a frontal lobe abscess due to aspergillosis. To the present, there have been a total of only 10 cases involving the central nervous system and these in the form of either an abscess or a granulomatous meningitis (Figure 12). There are perhaps some seven other cases reported which have involved the nervous system as a result of spread from adjacent bone or as a result of systemic infection. However, some of these cases are not too well authenticated. Recently, two cases were recorded, one in a 26-year-old soldier where there was a posterior fossalike tumor picture suspicioned but pathologically this proved to be a severe basilar and spinal granulomatous meningitis. The other case was that of a 10-month-old infant with a picture of basilar meningitis which pathologically revealed many aspergillus granulomata in the cortex(33).

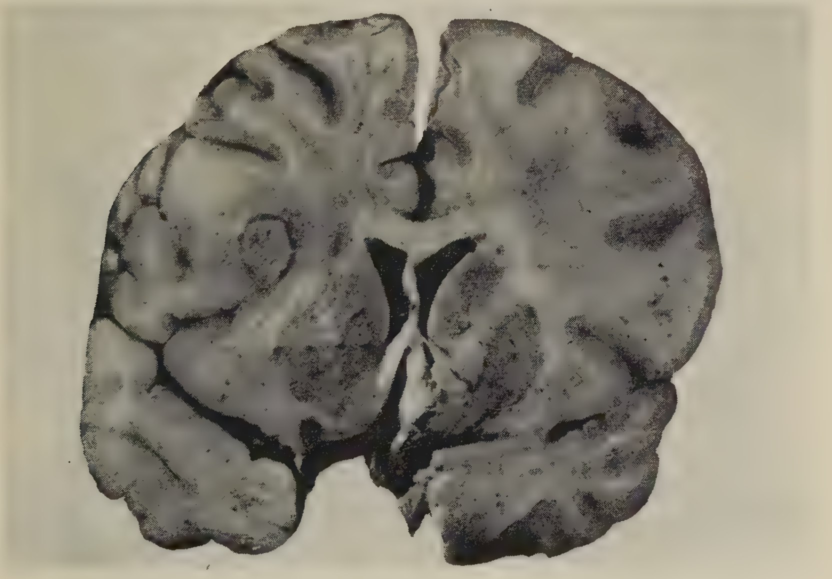

Figure 12
Gross section with *Aspergillus* abscess.

The organism in question is *Aspergillus fumigatus*, which appears as broken fragments of hyphae with numerous small (2 to 3) round, green spores (Figures 13, 14, 15).

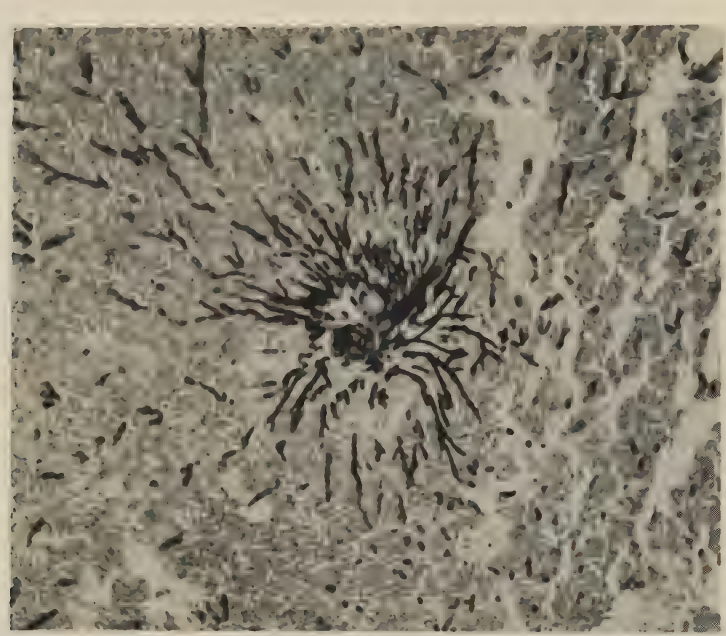

Figure 13
Small *Aspergillus* abscess in cerebrum. H & E × 125

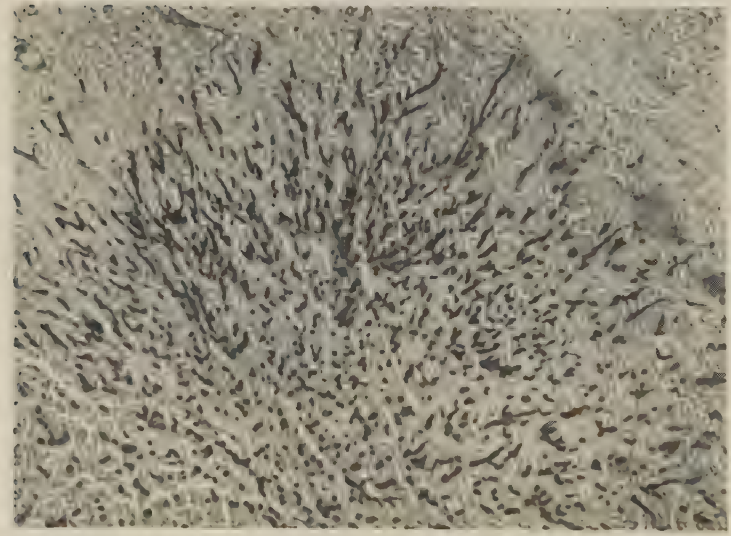

Figure 14
G.O. - Section through abscess in cerebrum. PAS × 125

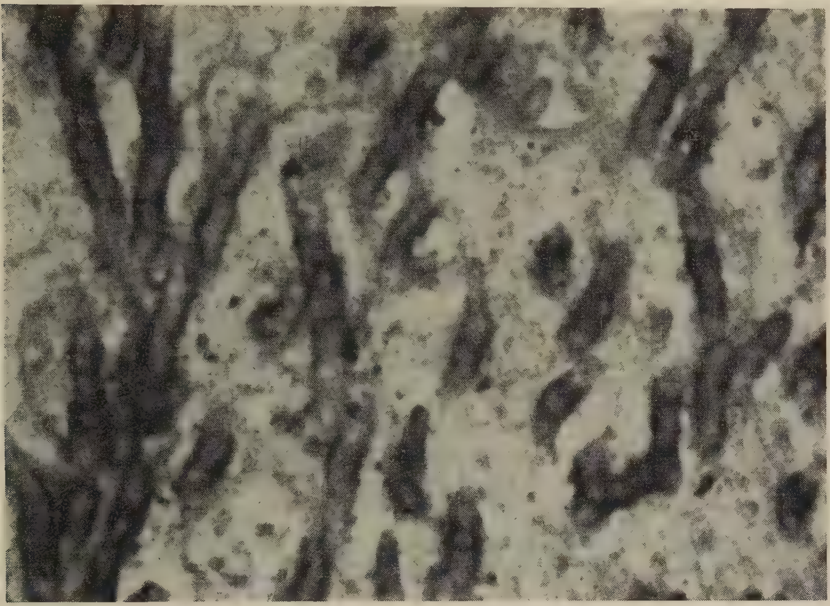

Figure 15
Aspergilli in brain abscess showing septa PAS × 500

Treatment:

At the present time, there is no specific treatment for this illness, and the management of choice still remains iodide therapy. Nystatin, the antifungal antibiotic produced by *Streptomyces noursei*, discovered by BROWN & HAZEN in 1951 and marketed under the trade name Mycostatin, has shown some promise in some isolated cases. Localized aspergillus abscesses are ordinarily drained and granulomatous areas are exised wherever possible.

CANDIDIASIS

Candidiasis, moniliasis, or thrush, is a disease process caused by the species of *Candida*, usually *C. albicans*. This is an acute or subacute infection in which the fungus may produce lesions of the mouth, bronchi, lungs, skin. There can result a septicemia with a concomitant endocarditis. As far as the central nervous system is concerned, a meningitis may be present. Distribution of this organism is world wide and all ages and both sexes are involved equally.

In 1831, LANGENBECK discovered this yeast-like fungus, *albicans*, in oral thrush. Later ROBIN described this organism under the name of *Oidium albicans* in 1853. Well over 170 synonyms exist to the present for this illness. The organism is believed to reach the meninges by way of the pharynx or even through the bloodstream. It has also been noted that secondary monileal infection is possible after prolonged use of antibiotics.

The clinical picture is that of a low grade meningitis. In the spinal fluid may appear a pleocytosis, not too high. The glucose and chloride content is diminished; the protein content elevated. The diagnosis is confirmed by the identification of the organism *Candida albicans*, a small round or oval yeast-like cell 2—6 μ in diameter. It is a budding, thin-walled structure attached to hyphae at various points of constriction.

Treatment:

At the present time, there is no specific treatment and general management consists of the usual supportative measures. Sodium iodide is still being used and Mysteclin, a new antifungal antibiotic, containing Mycostatin, is employed generally. Occasionally this is combined with the administration of other ordinary broad spectrum antibiotics. Mycostatin has also had some remarkable effects in some of these systemic monileal infections. Irradiation and steroids had been employed in many cases. S. L. ZIMMERMAN et al. reported a case of monileal meningitis, hitherto fatal, treated successfully with only streptomycin(34). Finally, an occasional case that has been reported in the recent past has had a measured amount of success with Amphotericin B.

SPOROTRICHOSIS

This is also a chronic infection which ordinarily begins as a subcutaneous nodule, usually at some site of injury. This progresses via the lymphatic channels and lymph nodes draining the area. These lesions occasionally occur on other exposed surfaces and very rarely is the infection systemic. Sporotrichosis occurs quite often in farmers, is world-wide in distribution and both sexes and all ages are susceptible. The organism involved is the *Sporotrichum schenckii.*

As far as the central nervous system is concerned, there is very rarely extension but there has been a case of meningitis reported by HYSLOP et al(35). Pathologically, there was seen a gelatinous form of meningitis at the base of the brain. Only 2 cells and 160 spores were seen in each cubic millimeter of spinal fluid. The oragnism is a delicate, branching, septate hyphae and there are spherical or oval conidia 2—6 μ in size.

Treatment:

The treatment of choice continues to be potassium iodide by mouth or intravenously. Stilbamadine, Thymol, Nystatin, Amphotericin B and Nepara have been used with some success. Occasionally surgery is indicated.

MUCORMYCOSIS

This is a rapidly progressive and usually fatal illness characterized by acute infection or vascular thrombosis caused by invasion of the walls and lumina of blood vessels by the organism in question. Diabetes mellitus appears to be a precipitating factor to infection and pulmonary mucormycosis in the diabetic is generally fatal. Occasionally this illness may be seen in leukemia and multiple myelomata, particularly after treatment with steroids or with X-ray. In the cental nervous system, Mucormycosis is represented by this triad: 1) uncontrollable diabetes, 2) orbital infections, 3) meningoencephalitis. Direct infection of the brain and meninges via the paranasal sinus and orbits is well established.

The organism is usually *Mucor mucedo.*

Treatment:

There is no known treatment as yet available for this infection. Some of the newer anti-fungal agents include Candicidin, Nepara and Eulicin (36), (37), (38).

HISTOPLASMOSIS

This is an illness endemic to the central and east central portion of the United States but cases are reported in various areas not only on this, but on other continents. It is caused by *Histoplasma capsulatum,* which is a diphasic organism growing as a mycelial saprophyte in soil and as a yeast-like organism in infected tissue. While it does not affect chickens, it seems to have a most unusual relationship to chicken excreta. Histoplasmosis as yet has not been proven to be spread from man to man (39). Although most classifications of this illness seem related to the pulmonary area, there are disseminated lesions which may reach the central nervous system. Only a few scattered reports are available.

Treatment:

The treatment of choice at present is Amphotericin B. 2-hydroxy stilbamadine is effective in some few cases. Actidione, Amebacide and Cycloserine have proven most ineffective as has Nystatin (40), (41). The sulfonamides appear to have some beneficial action in this illness (42). Combinations of Amphotericin B and sulfonamides have helped in a few scattered cases (43).

CHROMOBLASTOMYCOSIS

This name is misleading because the fungi in this group do not form blastospores or buds in tissue but they do have a characteristic brown color. Known also as verrucous dermatitis, Chromoblastomycosis is caused by a variety of fungi which cause formation of watery cutaneous nodules and these ultimately form prominent papillomatous vegetations which may or may not ulcerate. The lower extremities seem confined most of the time. This illness has been reported everywhre in the world except in Asia.

Mycotic brain abscess due to *Cladosporium trichoides* have been reported (44), (45). There was a fatal outcome in the first case but the second responded well to penicillin and sulfadiazine.

Conclusions

The hitherto bleak picture seen in fungus infections of the nervous system is brightening with the advent of newer and more powerful medications which are resulting in fungal and clinical arrests plus long remission periods. With this recent surge of interest in the management of these illnesses, we can now look ahead to continuing advances in the field of mycology.

References

1. Nickerson, W. J.: Medical Mycology, Ann. Rev. Microbiol. **7:** 245, 1953.
2. Boshes, L. D., Sherman, I. C., Hesser, C. J., Milzer, A. & MacLean, H.: Fungus Infections of the Central Nervous System, AMA Arch. Neurol. & Psychiat. **75:** 175—197, 1956.
3. Weidman, F. D. & Freeman, W.: India Ink in the Microscopic Study of Yeast Cells, JAMA **83:** 1163, 1924.
4. Mosberg, W. H., Jr. & Arnold, J. G., Jr.: Torulosis of the Central Nervous System: Review of the Literature and Report of 5 Cases, Ann. Int. Med, **32:** 1153, 1950.
5. Collins, V. P., Gellhorn, A. & Trimble, J. R.: Coincidence of Cryptococcosis and Diseases of the Reticulo-Endothelial and Lymphatic Systems, Cancer **4:** 883, 1951.
6. Rodger, R. C., Terry, L. L. & Binford, C. R.: Histoplasmosis, Cryptococcosis, and Tuberculosis Complicating Hodgkin's Disease: Report of a Case, Amer. J. Clin. Path. **21:** 153, 1951.
7. Zimmerman, L. E. & Rappaport, H.: Occurrence of Cryptococcosis in Patients with Malignant Diseases of the Reticuloendothelial System, Amer. J. Clin. Path. **24:** 1050, 1954.
8. Martin, H. & Patberg, F.: Torulosis of the Brain, AMA Arch. Neurol. & Psychiat. **62:** 679, 1949.
9. Wilson, H. M. & Duryea, G. W.: Cryptococcus Meningitis (Torulosis) Treated with a New Antibiotic, Actidione, AMA Arch. Neurol. & Psychiat. **66:** 470, 1951.
10. Ley, A., Jacas, R. & Oliveras, C.: Torula Granuloma of Cervical Spinal Cord, J. Neurosurg. **8:** 327, 1951.
11. Procknow, J. & Loosli, C.: Treatment of the Deep Mycoses, AMA Arch. Int. Med. **101:** 765, 1958.
12. Gold, W., Stout, H. A., Pagano, J. F. & Donovick, R.: Amphotericins A and B, Antifungal Antibiotics Produced by a Streptomycesti: I. In Vitro Studies, Antibiotics Ann., 579, 1955.
13. Vanderputte, J., Wachtel, J. L. & Stiller, E. T.: Amphotericins A and B, Antifungal Antibiotics Produced by a Streptomyceti: II. The Isolation and Properties of the Crystalline Amphotericins, Antibiotic Ann., 587, 1955.

14. Biddle, A. & Koenig, R.: An Agent Effective Against Cryptococcosis of the Central Nervous System, AMA Arch. Int. Med. **102:** 801—05, Nov.,1958.
15. Kligman, A. M. & Mescon, H.: The Periodic-Acid-Schiff Stain for the Demonstration of Fungi in Animal Tissues, J. Bact. **60:** 415, 1950.
16. Klapper, M. S., Smith, D. T. & Conant, N. F.: Disseminated Coccidioidomycosis Apparently Cured with Amphotericin B, JAMA **167:** 463, 1958.
17. Forbus, W. B.: Coccidioidomycosis: Study of 95 Cases of Disseminated Type with Special Reference to Pathogenesis of the Disease, Mil. Surgeon **99:** 653, 1946.
18. Schlumberger, H. G.: A Fatal Case of Cerebral Coccidioidomycosis with Culture Studies, Amer. J. Med. Sci. **209:** 843, 1945.
19. Abbott, K. M. and Cutler, O. I.: Chronic Coccidioidal Meningites, Arch. Path. **21:** 320, 1936.
20. Jenkins, V. E. & Postlewaite, J. C.: Coccidioidal Meningitis: Report of 4 Cases, with Necropsy Findings in 3 Cases, Ann. Int. Med. **35:** 1084, 1951.
21. Rand, C. W.: Coccidioidal Granuloma: Report of 2 Cases Simulating Tumor of the Spinal Cord, AMA Arch. Neurol. & Psychiat. **23:** 502, 1930.
22. Norman, D. D. & Miller, Z. R.: Coccidioidomycosis of the Central Nervous System: A Case of 10 Years' Duration, Neurology **4:** 713, 1954.
23. Litman, M., Horowitz, P. & Swadey, J: Coccidioidomycosis and its Treatment with Amphotericin B, Amer. J. Med. 568, 1958.
24. Kuchner, D., Snapper, U., Senders, M. & McMillan, S.: Aminostilbamidine a Fungistatic Agent, J. Invest. Derm. **28:** 69, 1957.
25. Harvey, J., Cantrell, J. & Fisher, A.: Actinomycosis: Recognition and Treatment, Ann. Int. Med. **46:** 5, 1957.
26. Jacobson, J. Robert & Cloward, Ralph B.: Actinomycosis of the Central Nervous System, JAMA **137:** 769, 1948.
27. Bates, M. & Cruchshank, G.: Thoracic Actinomycosis, Thorax **12:** 99, 1957.
28. Peabody, J. & Seabury, J.: Actinomycosis and Nocardiosis, J. Chron. Dis. **5:** 347, 1957.
29. Rankin, J. & Javid: Nocardiosis of the Central Nervous System, Neurology **5:** 815, 1955.
30. Runyan, E. H.: Nocardia Asteroides — Studies on its Pathogenicity and Drug Sensitivities, J. Lab. Clin. Med. **37:** 713, 1951.
31. Smith, D. T.: Therapy for Pulmonary and Systemic Fungus Diseases, Post Grad. Med. **20:** 181, 1956.
32. Moniz, E. & Loff, R.: Aspergillose Cérébrale, Presse Med. **39:** 273, 1931.
33. Iyer, S., Dodge, P. R. & Adams, R. D.: Two Cases of Aspergillus Infection of the Central Nervous System, Neurol. Neurosurg. & Psychiat. **15**: 152, 1952.
34. Zimmerman, S. L., Frutchey, L. & Gibbs, J. H.: Meningitis Due to Candida (Monilia) Albicans with Recovery, JAMA **135:** 145, 1947.
35. Hyslop, G. H., Neal, J. B., Kraus, W. M. & Hillman, O. A.: A case of Sporotrichosis Meningitis, Amer. J. Med. Sci. **172:** 726, 1926.
36. Kligman, A. & Lewis, F.: Action of Candicidin on Pathogenic Fungi, Proc. Soc. exp. Biol. & Med. **82:** 399, 1953.
37. Oswald, E., Reedy, R. & Randall, W.: (Nepera), Antibiotics Annual, 1955—1956.
38. Charney, J., Machlowitz, R., McCarthy, F., Reitbowski, G. & Tytell, A.: Eulicin, a New Antifungal, Antibiotics Annual, 1955—1956.
39. Emmons, G.: Histoplasmosis, Public Health Reports 72 (11) Nov. 1957, pp. 981—988.
40. Lehan, P., Yates, J., Brasher, C., Larsh, H. & Furculow, M.: Experiences with the Treatment of 60 Deep Mycotic Infections, Dis. Chest **32:** 597, 1957.
41. Terry, R. & Matthew, J.: Histoplasmosis: Treatment of 2 Cases with B-diethylanine ethyl Fencholate, Dis. Chest **30:** 570, 1956.
42. Louria, D. & Feder, N.: Sulfonamides in Experimental Histoplasmosis, Antibiotics **7:** 471, 1957.
43. Baum, G., Ruchel, H. & Schwarz, J.: Treatment of Experimental Histoplasmosis, Antibiotics **7:** 472, 1957.

44. King, A. B. & Collette, T. S.: Brain Abscess Due to Cladosporium Trichoides, Bull. Johns Hopkins Hosp. **91:** 298, 1952.
45. Binford, C. H., Thompson, R. K. & Gorham, M. E.: Mycotic Brain Abscess Due to *Cladosporium Trichoides,* a New Species, Amer. J. Clin. Path. **22:** 535, 1952.

ADDITIONS TO OUR KNOWLEDGE OF ASCOMYCETES OF INDIA - I.

T. S. Viswanathan & S. T. Tilak

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(4.V.1960)

During the course of their mycological collections in Poona and neighbouring places the authors collected a number of Ascomyceteous fungi which are proposed to be published in a series of papers, the present one being the first. Here are described six new species, with the genus *Sydowia* being represented for the first time in the Indian Fungi, on the basis of comparative morphology and host relationship. The type specimens have been deposited in the herbaria of the Commonwealth Mycological Institute, Kew, England and the Indian Agricultural Research Institute, New Delhi, India.

We are grateful to Prof. M. N. Kamat for his advice and encouragement to carry out the present work and critically reading through the manuscript. We thank Dr. S. P. Agharkar, Director, M.A.C.S. Laboratory for the laboratory facilities offered and Rev. Father Santapau, S. J., for kindly translating the diagnosis into Latin. Our thanks are also due to Dr. Emil Müller of Swiss Federal Institute of Technology, Zurich and Von Arx of Phytopathological Laboratory, Baarn, the Netherlands for their ready help in the identification of some of the Ascomycetes.

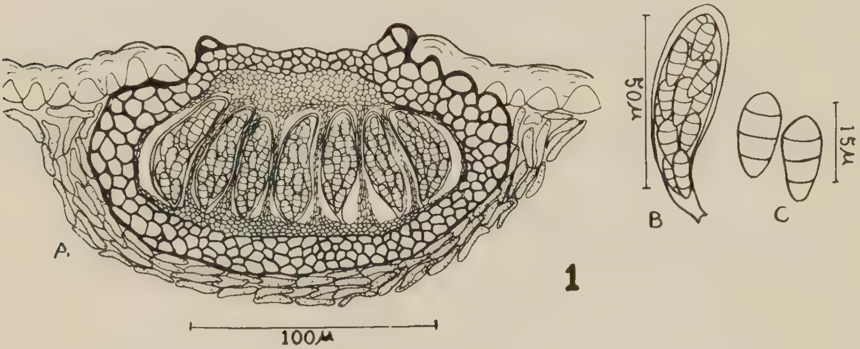
1. *Sydowia agharkari* Viswanathan sp. nov.

Perithecia subepidermal, immersed within the host tissue, globose or frequently depressed, 97.5 — 165.0 μ, usually single but sometimes 2 to 4 in a group, perithecial wall composed of brown-coloured cells of irregular shape, ostiolate. Asci bitunicate, clavate, each ascus contained within a locule, arising from cushion-like basal polygonal parenchymatous cells, 39.0 — 51.0 μ in length and 15.5 — 19.5 μ in breadth, asci polyspored. Ascospores spindle shaped, hyaline, 4-celled, 15.0 — 17.0 X 5.0 — 6.8 μ. Paraphyses absent.

On stems of *Jasminum primulinum* Hemsl., Law College, Poona, 9-2-1959. Leg. T. S. Viswanathan.

Sydowia agharkari Viswanathan sp. nov.

Perithecia subepidermalia, immersa in textus plantae hospitis, globosa vel saepius depressa, 97.5—165.0 μ, vulgo unica, sed nonnumquam bina - quaterna, parietibus constantibus e cellulis brun-



A — Section through Perithecium
B — Ascus C — Ascospores

Fig. 1. *Sydowia agharkari*

neis formae irregularis, ostiolata. Asci bitunicati, clavati, singuli positi in loculo, emergente ex cellulis basalibus polygonalibus parenchymaticis pulvino similibus, 39.0 — 51.0 μ longi, 15. 5 — 19.5 μ lati, polyspori. Ascosporae fusiformes, hyalinae, 4-cellulatae, 15.0—17.0 X 5.0 — 6.8 μ Paraphyses nullae.

In culmis Jasmini primulini Hemsl. ad Law College in urbe Poona die 9 februarii anni 1959, leg. T. S. VISWANATHAN.

This species has been described after Dr. S. P. AGHARKAR, Director, M.A.C.S. Laboratory, Poona in recognition of his services to Indian Botany.

2. *Didymella muelleri* VISWANATHAN sp. nov.

Perithecia subepidermal, immersed within the host tissue, globose or frequently depressed, 253.5 — 338.0 μ, perithecial wall composed

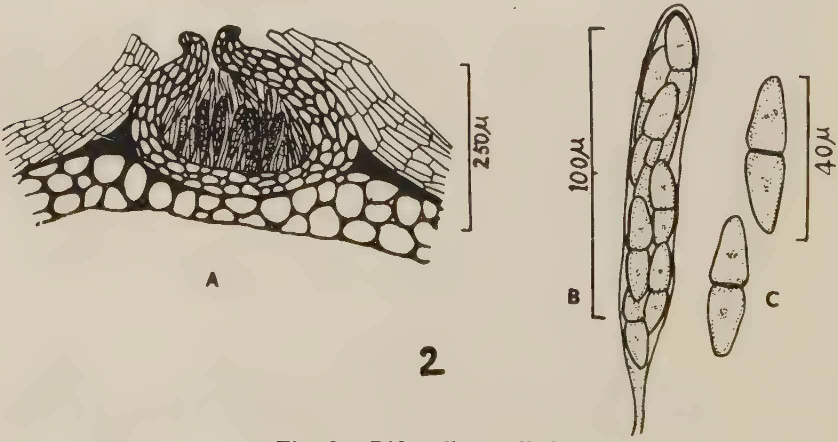


Fig. 2. *Didymella muelleri*

of 3—4 layers of brownish cells, ostiolate. Asci numerous, cylindrical, 97.5 — 117.0 X 15.6 — 19.5 μ, 8-spored. Ascospores hyaline, 2-celled, biseriate, 30.6 — 34.0 X 6.8 — 8.5 μ. Paraphysoids numerous, filiform.

On dried stems of *Duranta plumeri* FACQ., Poona, 9-2-1959. Leg. T. S. VISWANATHAN.

Didymella muelleri VISWANATHAN sp. nov.

Perithecia subepidermalia, immersa in textus plantae hospitis, globosa vel saepius depressa, 253.5 — 338.0 μ, parietibus constantibus e serie terna quaternave cellularum brunnearum, ostiolata. Asci plures, cylindrici, 97.5 — 117.0 X 15.6 — 19.5 μ. Ascosporae hyalinae, 2-cellulatae, biseriatae, 30.6 — 34. 0 X 6.8 — 8.5 μ. Paraphysoidea plurima, filiformia.

On culmis siccis Durantae plumieri ad Poona, die 9 februarii anni 1959. Leg. T. S. VISWANATHAN.

This species has been described after Dr. EMIL MÜLLER of Zurich in recognition of his outstanding contribution towards taxonomical work on Ascomycetous fungi.

3. *Massarina jasminicola* VISWANATHAN sp. nov.

Perithecia immersed within the host tissue, globose, perithecial wall 4-6 cells thick, brown in colour, 117.0 — 183.0 μ, ostiolate. Asci numerous, cylindrical to clavate, 58.5 — 78.0 μ 8-spored. Ascospores hyaline, 2-celled, 18.5 — 22.0 X 6.8 — 8.5 μ, ascospores surrounded by mucous sheath. Paraphysis numerous, filiform.

On dried stems of *Jasminum malabaricum* WALL., Poona, 9-2-1959 Leg. T. S. VISWANATHAN.

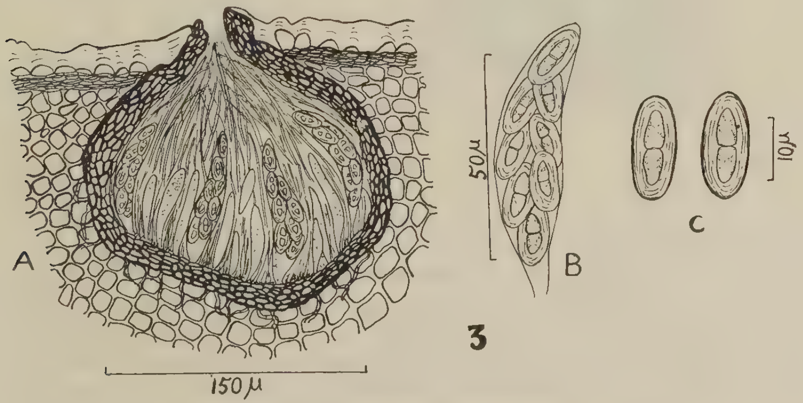


Fig. 3. *Massarina jasminicola*

Massarina jasminicola VISWANATHAN sp. nov.

Perithecia immersa in textus plantae hospitis, globose, pariete 4—6 cellulis crass, brunnea, 117.0 — 183.0 μ, ostiolata. Asci plurimi, cylindrici vel clavati, 58.5 — 78.0 μ, octospori. Ascosporae hyalinae, 2-cellulatae, 18.5 — 22.0 X 6.8 — 8.5 μ, circumdatae vagina mucosa. Paraphyses plurimae, filiformes.

In culmis siccis Jasmini malabarici Wall, ad Poona, die 9 februarii, anni 1959, Leg. T. S. VISWANATHAN.

4. *Ophiobolus helianthi* TILAK sp. nov.

Perithecia single, superficial with prominent beak more or less globose, 150—200 X 165 — 250 μ. Ostiolate, wall 2—4 cells thick outermost cells heavily thickened, brown neck 60 — 99 X 43 — 84 μ.

Asci numerous, originate from basal layer, pedicellate, 8-spored, thin walled, unitunicate, cylindrical, tubular, tapering at base and rounded at apex, 76 — 88 X 8 — 12 μ.

Ascospores filiform, septate, tapering at end 68 — 76 X 1.7 — 2 μ. Paraphyses filiform interspersed with asci. Periphysis lining the neck.

On dried stems of *Helianthus annuus* L., Law College, Poona, July-August 1959. Leg. S. T. TILAK.

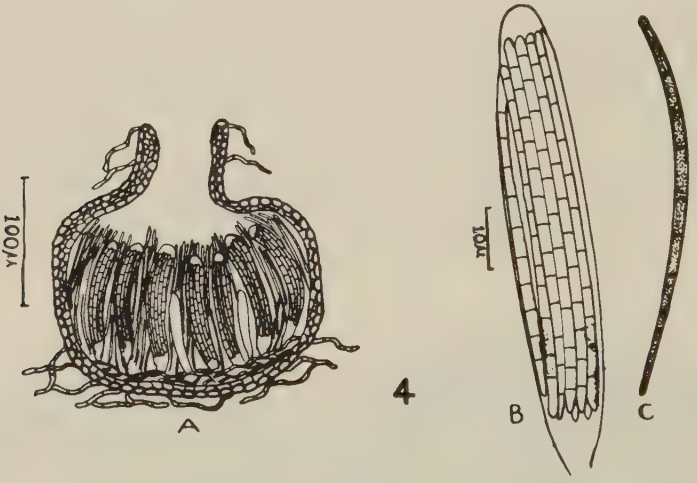


Fig. 4. *Ophiobolus helianthi.*

Ophiobolus helianthi TILAK sp. nov.

Perithecia singula, superficialia rostro prominente, plus minusve globosa, 150 — 200 X 165 — 250 μ, ostiolata, parietibus crassis 2—4 cellulis, quarum exteriores sunt ponderose crassae, collo brunneo, 60 — 99 X 43 — 83 μ. Asci plurimi, originem ducentes e serie basali, pedicellati, octospori, tenuibus parietibus, uni-tunicati, cylindrici, tubulares, fastigati ad basin, rotundati ad apicem, 76 —

88 X 8 — 12 μ. Ascosporae filiformes, septatae, fastigatae ad apices, 68 — 76 X 1.7 — 2 μ. Paraphyses filiformes, ascis intermixtae. Periphyses collum implentes.

In culmis siccis Helianthi annui L. ad Law College, in urbe Poona, mensibus julio-augusto anni 1959, leg. S. T. TILAK.

5. *Leptosphaeria helianthi* TILAK sp. nov..

Perithecia single, embedded in host tissue, globose, ostiolate, protruding out of epidermis.

Asci numerous, originating from basal layer, cylindrical, bitunicate, 115 — 148 X 32 — 45 μ, 8-spored.

Ascospores fuscoid, curved, primarily hyaline but later on turning brownish, septate, septa horizontal, 4 to 6, 37 — 44 X 8—10 μ.

On dried stems of *Helianthus annuus* L. Law College, Poona, July-August 1959. Leg. S. T. TILAK.

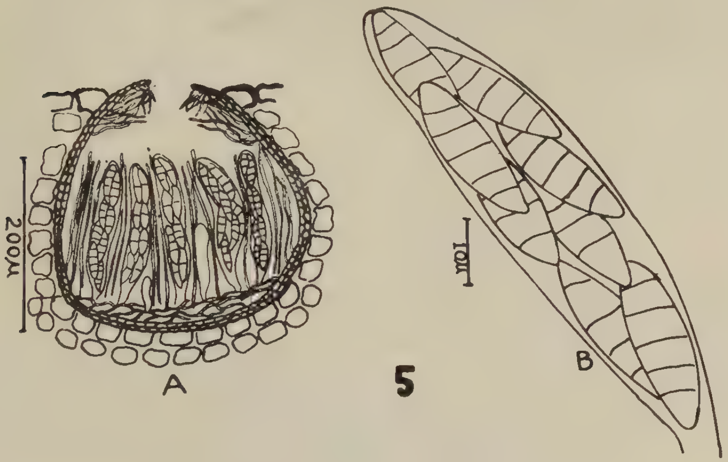


Fig. 5. *Leptosphaeria helianthi.*

Leptosphaeria helianthi TILAK sp. nov.

Perithecia singula immersa in textus plantae hospitis, globosa, ostiolata, ex epidermate protrudentia. Asci plurimi, originem ducentes ex serie basali, cylindrici, bitunicati, 115—148 X 32—45 μ, octospori. Ascosporae fuscoideae, curvatae, primo hyalinae, demum brunneolae, septatae, septis horizontalibus, 4—6, 37—44 X 8—10 μ.

In culmis siccis Helianthi annui L. ad Law College, in urbe Poona, mensibus julio-augusto anni 1959, leg. S. T. TILAK.

6. *Chaetothyrina mundkuri* TILAK sp. nov.

Perithecia superficial, hypophyllous, dark brown, single, setose, setae 3—4 celled, 38—57 μ long, slightly bulged at base, 50—80 X 100—150 μ.

Asci numerous, ellipsoid to oblong 60—75 X 8—12 μ, bitunicate, 8-spored, paraphysoids intermingled with asci.

Ascospores hyaline, 2-celled, slightly constricted at septum, biseriate, but later more compactly arranged 17 — 20 X 3— 5 μ.

On living leaves of *Chonemorpha fragrans* (Moon) Alston, Khandala, 1958. Leg. S. T. Tilak.

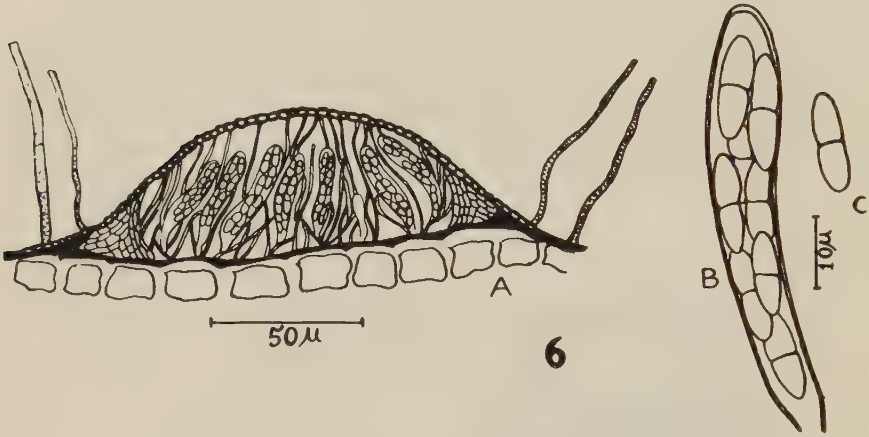


Fig. 6. *Chaetothyrina mundkuri.*

Chaetothyrina mundkuri Tilak sp. nov.

Perithecia superficialia, hypophylla, fusce brunnea, singula. setosa, setis 3 — 4-cellulatis, 38 — 57 μ longis, tenuiter tumescentibus ad basin, 50 — 80 X 100 — 150 μ. Asci plurimi, ellipsoidei vel oblongi, 60 — 75 X 8 — 12 μ, bitunicati, octospiri, paraphysoideis intermixti. Ascosporae hyalinae, bicellulatae, paulum constrictae ad septa, biseriatea, sed demum arctius dispositae, 17 — 20 X 3 — 5 μ.

In foliis viventibus Chenemorphae fragrantis (Moon) Alst. ad Khandala, 1958. Leg. S. T. Tilak.

This species has been described after late Dr. B. B. Mundkur in recognition of his outstanding contribution towards Indian Mycology.

STIGMATOMYCOSIS IN INDONESIA

BY

K. B. Boedijn (*The Hague, The Netherlands*)

(2.VI.1960)

Under the name Stigmatomycosis a disease of plants is designated, caused by fungi, which are inoculated in them through the agency of insects. Mostly bugs (Rhynchota) are responsible for the transmission of the fungi, by means of their sucking apparatus. From fruits injured in this way, the following species could be isolated.

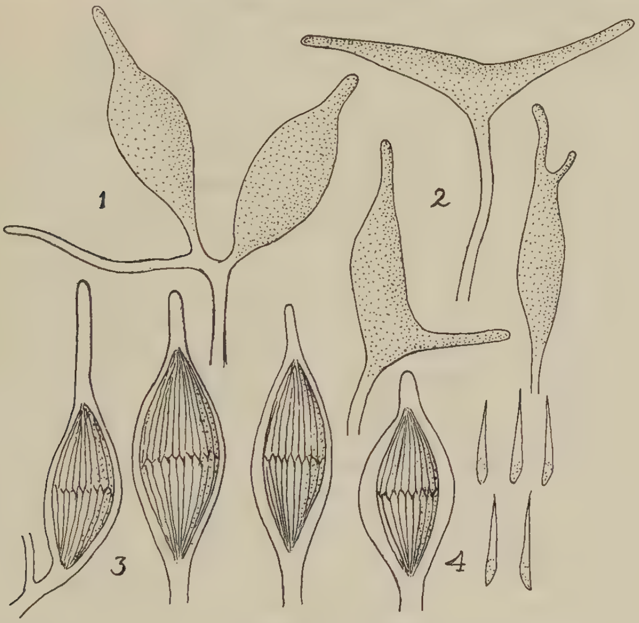


Fig. 1. *Eremothecium cymbalariae.*
1 young asci. 2 Aberrant asci. 3 mature asci. 4 ascospores.

Eremothecium cymbalariae Borzi Bull. Soc. bot. Ital. 20, 455. 1888.

Colonies on toge agar after about 1 month 6 cm in diameter. They are circular with rather sharp, weakly undulating border, dirty white, semitranslucent, centre mostly fluffy. Under a handlens colony finely radially striate and concentrically zonate.

Mycelium richly developed, the threads subdichotomously branched, usually winding and undulating, with much protoplasm and small oildrops, but few septae; 1½—5 μ in diam. No budding cells ever formed. At the end of the branches the asci are originating. They are formed mostly a short distance from the top, so that they are provided here with a more or less long rostrum-like appendage.

The shape is typical fusiform; 34—51 × 12—25 μ, rostrum 5—38 × 2—3½ μ, containing about 30 or more spores. The spores arranged in two conical shaped bundles, which are dovetailing with the broad ends, so as to from a fusiform body. Spores hyaline, elongated, one pole acuminated, the other gradually attenuated into a thin, sharp point; 19—25 × 1½—3 μ, apparently twocelled, because the broader part contains much protoplasm, which can be demonstrated by staining, whereas the attenuated end remains nearly empty.

It seems, that the asci are opening in the middle, either by fissure or by deliquescence.

Java, Bogor, isolated from Citrus fruits.

Nematospora coryli PEGLION Zbl. Bakt. (2 Abt.) 7, 754. 1901.

Colonies on toge agar after a month about 5 cm in diameter, white till dirty white, border typical ciliate, surface dull, weakly humid, finely radially plicated under a handlens.

Mycelium well developed, branched and septated, the separate threads 2—5 μ broad. In the older hyphae, intercalar and sometimes also apical swellings are formed on rather regular distances, often directly under a crosswall. They are clubshaped, oval till globose 14—31 × 7—20 μ, Lateral on the hyphae, sometimes on the swellings, budding cells 10—17 × 4—7 μ are originating, either directly or on a stem-like cell, usually under or above a septum. Soon they are shed, mostly together with the supporting cell and they are now increasing in size, measuring 20—24 × 7—10 μ, whereas they continue to sprout. On the end of main and lateral branches short rows of globose arthrospores 17—31 μ in diam. are formed, which soon are set free and multiply profusely by budding. They look rather empty, containing mostly only a small lump of substance. The asci formed at the end of threads, sometimes intercalary, are ellipsoid till cylindrical, 88—109 × 9—17 μ, containing 8 spores, which are arranged in two bundles of 4. Occasionally a 4 spored ascus is seen. All mature asci are soon set free. Ascospores narrow fusiform, hyaline, twocelled, 48—59 × 3—4 μ, one cell acuminated, the other which contains much more protoplasm is ending in a long flagelliform thread, 24—54 μ long. Those threads, which are easily seen on the free spores, are hardly perceptible on the spores in the ascus.

Just under the crosswall in the spore, an indistinct flat, onesided swelling may be observed.

Java, Bogor, isolated from Citrus fruits.

Both genera undoubtedly related to one another, are belonging to the Hemiascomycetes. Whereas however *Nematospora* is mostly placed in the order Endomycetales, *Eremothecium*, due to the absence of yeastlike cells is excluded from this order, together with *Spermophthora* likewise a fungus of Stigmatomycosis.

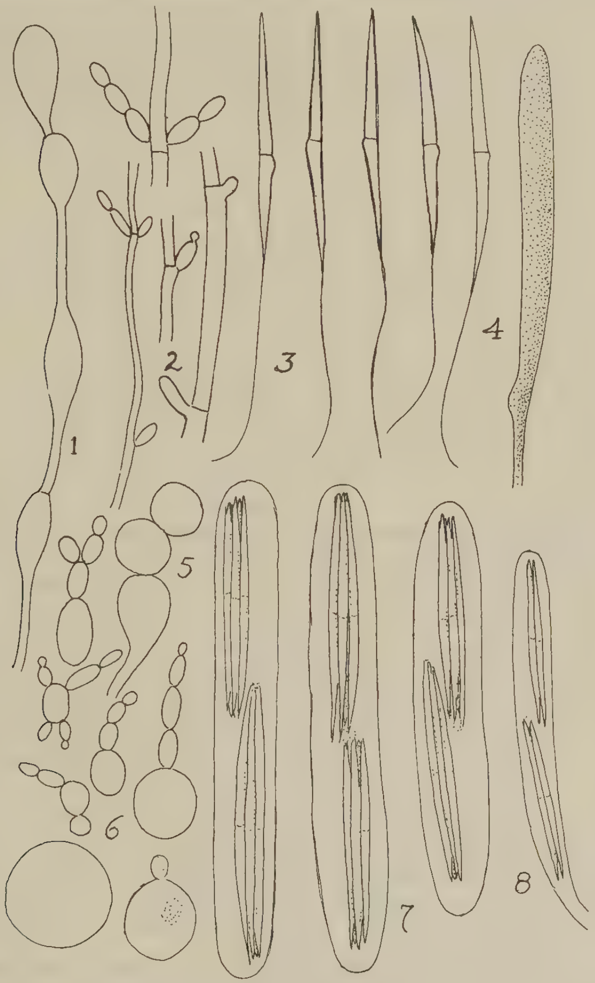


Fig. 2. *Nematospora coryli.*
1 thread with swellings. 2 formation of budding cells. 3 ascospores. 4 young ascus. 5 formation of arthrospores. 6 Budding cells. 7 mature asci. 8 four spored ascus.

Some authors have erected a subfamily Nematosporoideae in the family Endomycetaceae to accomodate *Nematospora*, *Monosporella* and *Coccidiascus*. The two last named genera, wholly yeastlike, but with the same kind of ascospores as in *Nematospora*, are only found as parasites in lower animals. Others have established the family Spermophthoraceae, to include the genera *Spermophthora, Eremothecium, Ashbya,* and *Nematospora.* Sometimes even an order Spermophthorales is proposed.

At present it seems advisable to accept a separate family Spermophthoraceae next to the family Endomycetaceae.

The chief character of this family is to be found in the ascospores, which are typical elongated till needle-shaped. Most species possess a well developed mycelium. Budding cells are absent in a few, sparingly present in others and increasing in numbers in species with poorly developed mycelium, till in reduced forms such as *Monosporella* and *Coccidiascus,* only yeast cells are to be found.

Taking into account the above mentioned characters, the following genera may be placed in our family;

Spermophthoraceae.

Spermophthora Ashby et Nowell, Ann. Bot. 40, 72. 1926
Eremothecium Borzi, Bull. Soc. bot. Ital. 20, 455. 1888.
Crebrothecium Routien, Mycologia 41, 184. 1949.
Ashbya Guilliermond, Rev. gén. Bot. 40, 609. 1928.
Nematospora Peglion, zbl. Bakt. (2 Abt.) 7, 754. 1901.
Coccidiascus Chatton, C. R. Soc. Biol. 75, 117. 1913.
Monosporella Keilin, Parasitology 12. 83. 1920.

Literature

Arnaud, G. (1913) Sur le genre Eremothecium Borzi. Bull. Soc. mycol. France **29,** 572—576, 1 fig. 1 pl.

Ashby, S. F. & Nowell, W. (1926) The fungi of Stigmatomycosis. Ann. Bot. **40,** 69—84, 2 pl.

Fawcett, H. S. (1929) Nematospora on Pomegranates, Citrus and Cotton in California. Phytopathology **19,** 479—482, 1 fig.

Guilliermond, A. (1927) Cytologie et sexualité du Spermophthora Gossypii. C. R. Acad. Sci. Paris **184,** 1189—1192.

—, (1928) Recherches sur quelques Ascomycètes inférieurs isolés de la stigmatomycose des grains de cotonnier. Essai sur la phylogénie des Ascomycètes. Rev gén. Bot. **40,** 328—342, 397—414, 474—485, 555—574, 606—624, 690—704. 46 fig. 2 pl.

—, (1928) Quelques faits nouveaux relatifs au développement du Spermophthora Gossypii. C. R. Acad. Sci., Paris p. 161—163.

—, (1936) L'Eremothecium Ashbyii, nouveau champignon parasite des capsulesdu cotonnier. Rev. Mycol. **1,** 115—156, 25 fig. 2 pl.

Guilliermond, A., Fontaine, M. & Raffy, A. (1935) Sur l'existence dans l'Eremothecium Ashbyii d'un pigment jaune, se rapportant au groupe des flavines. C. R. Acad. Sci., Paris **201,** 1077.

Kobayashi, Y. (1955) On the Spermophthorales, an order of Protoascomycetes. Nagaoa **5,** 77—120, 24 fig. (in Japanese language).

Lee, H. A. (1924) Dry rot of Citrus fruits caused by a Nematospora species. Philippine J. Sci. **24,** 719—733, 1 fig. 2 pl.

Lodder, J. & Kreger van rij, N. J. W. (1952) The yeasts, a taxonomic study. XI and 713 pp. 268 fig. Amsterdam.

Manuel, J. (1938) Sur la formation de l'asque de Nematospora coryli après un phénomène sexuel. C. R. Acad. Sci., Paris **207,** 1241—1243, 11 fig.

Pridham, Th. G. & Raper, K. B. (1950) Ashbya Gossypii — its significance in nature and in the laboratory. Mycologia **42,** 603—623, 4 fig.

Routien, J. B. (1949) Crebrothecium Ashbyii. Mycologia **41,** 183—185.

Wingard, S. A. (1925) Studies on the pathogenicity, morphology and cytology of Nematospora phaseoli. Bull. Torrey Bot. Club **52,** 249—290, 3 pl.

A PHYSIOLOGICAL STUDY ON THE TOXICITY OF CULTURE FILTRATE OF HELMINTHOSPORIUM SATIVUM P.K. & P.

BY

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(13.VI.1960)

It has been shown in many instances (e.g. HAYMAKER, 1928; ZENTMYER & HORSFALL, 1942; DIMOND, 1947; WHEELER & LUKE, 1954; and CEPONIS & FRIEDMAN, 1959), that symptoms of a disease could be reproduced by using the culture filtrate of the causal organism instead of inoculation with infecting spores. It was deduced in these cases that the toxic substance or substances responsible for causing the particular disease symptoms and which are produced *in vivo* are probably present in the culture filtrate.

It could also be shown (e.g. WHITE & WOLF, 1954, and PRINGLE & BRAUN, 1957) that the toxicity of such filtrate may be tested or even assayed by the extent to which these filtrates are capable of causing wilt to, or reducing root growth of, test plants.

However, by preliminary experiments done by the second author (unpublished work) at the Phytopathological Laboratory W.C.S., Baarn, Holland, it was found that the symptoms of the leaf-spot disease of barley caused by *Helminthosporium sativum* could be reproduced by using the culture filtrate of the fungus when grown on Fries' No 3 medium (composition given in text) for 30 days. It is therefore suggested that the toxin produced by the fungus and which is responsible for causing the particular symptoms of the disease that appear naturally on diseased plants must have been secreted in the culture media. It is interesting to recall here that DIMOND (1947) identified a polysaccharide and an alcohol-soluble component in the culture filtrate of *Graphium ulmi*, the causal organism of the Dutch elm disease. He further found that both components together are capable of producing the particular symptoms of the disease. PRINGLE & BRAUN (1957), on the other hand, succeeded in isolating a toxic substance capable of causing the typical symptoms of the blight disease of oats caused by *Helminthosporium victoriae*, from the culture filtrate of the fungus. They further stated that the active material in the toxin appears to be polypeptide in nature.

It was found desirable in the present investigation to try to throw some light on the nature of the toxic agent present in the fungal filtrate by running parallel experiments for studying the nitrogen metabolism of the fungus on the one hand and thewilt -inducing capacity of its culture filtrate on the other hand.

MATERIALS AND METHODS

The fungus used in the present investigation was *Helminthosporium sativum* P.K. & P., the causal agent of the leaf-spot disease of barley, obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland.

Composition of Media and Procedure.

The fungus was first grown on slants of Dox's medium made up of sucrose, sodium nitrate, potassium acid phosphate, potassium chloride, magnesium sulphate, and ferrous sulphate. Spore suspension was prepared from 15 day-old slant cultures and distributed in the culture flasks containing the culture medium. The basal medium used was a modified Fries No 3 medium of the following composition (as given by PRINGLE & BRAUN (1957) except for the nitrogen source): KH_2PO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; NaCl, 0.1 g; $CaCl_2.2H_2O$, 0.13 g; sucrose, 30 g; $MnSO_4.7H_2O$, 1.0 mg; boric acid, 1.0 mg; $CuSO_4$, 0.1 mg; $ZnSO_4.7H_2O$, 0.1 mg; $FeSO_4.7H_2O$, 20 mg; and distilled water to make 1000 ml. The medium was completed by adding the nitrogen source at 1.15 g N/1 in the form of sodium nitrate (7 g/1) or ammonium chloride (4.39 g/1) or a mixture of sodium nitrate (3.5 g/1) and ammonium chloride (2.19 g/1) of equivalent amounts of nitrogen.

Three media were thus prepared and were distributed in 50 ml quantities to 500 ml conical flasks. The three sets of culture flasks were then plugged and autoclaved at 10 lb for 15 minutes. After cooling and inoculation with the spore suspension, they were incubated at 25°C. After 4, 7, 10, 16, 22, 28 and 34 days, quadruplicate sets of flasks were withdrawn, the mycelial mats were separated by dry filtration and the filtrates were used for measuring the wilt-inducing capacity and for nitrogen analysis.

Methods of Estimation.

For measuring the wilt-inducing capacity of the culture filtrates, 15-day-old seedlings of lupin (*Lupinus termis*) were used. Lupin seedlings proved to be better, in this connection, than seedlings of cotton or broad beans; they were more sensitive to the toxin action due to their more or less fleshy nature. The seedlings were dipped each in 5 ml of the filtrate at full strength, and the decrease in fresh weight after 24 and 48 hours was then calculated. Seedlings were also dipped in tap water as control, and these were used for calculating the percentage difference due to filtrate toxicity.

For nitrogen analysis, a micro-Kjeldahl method was used. The nitrate was first reduced with reduced iron and sulphuric-salicylic acid mixture before digestion. Steam distillation of ammonia was carried out in the apparatus described by SCANDRETT (1953).

RESULTS

The drift in the nitrogen content of the culture media and the wilt-inducing capacity of the fungal filtrates were followed using the three sets of cultures, namely, the nitrate, the ammonium, and the nitrate-ammonium cultures, as follows:

1. The nitrate cultures.

The total nitrogen content of the nitrate media (Figure 1) decreased steeply and progressively until a minimum was reached on the 7th day. This was followed by the reappearance of nitrogen in the external media. It further accumulated and reached a maximum on the 28th day. A slight drop was later observed by the end of the experiment. The phenomenon of escape of nitrogenous compounds from the mycelial cells into the culture media has been previously recorded by several workers such as HOCKENHULL (1946), MORTON & BROADBENT (1955), v. HOFSTEN (1956) and NAGUIB (1959).

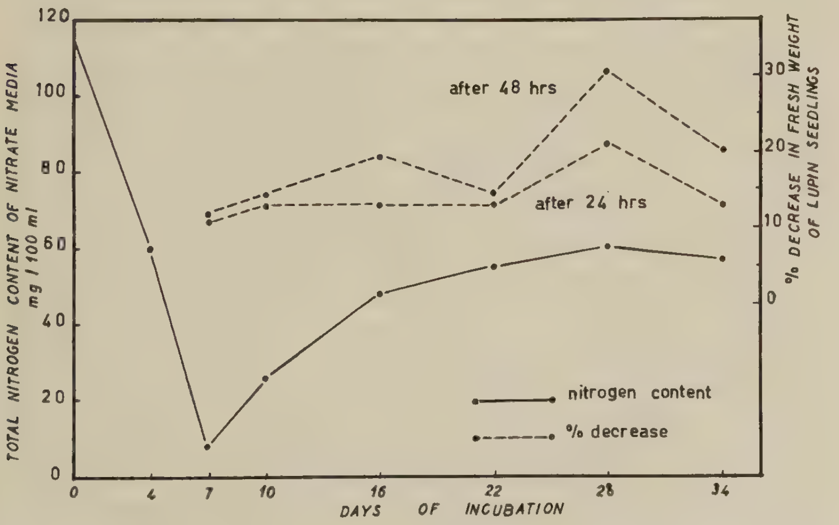

Fig. 1. Change in the total nitrogen content of the nitrate media during the incubation period, and the percentage decrease in fresh weight of lupin seedlings, when dipped for 24 and 48 hours, in culture filtrates obtained at corresponding intervals.

Results of the wilt-inducing capacity of the culture filtrate are also represented in Figure 1. They show that the toxicity of the filtrate increased gradually at the beginning and then was maintained constant at the rate of 13% decrease in fresh weight from the 10th until the 22nd day. Further on, a marked increase in toxicity was noted up to the 28th day, followed by a gradual decline. The drop in fresh weight of lupin seedlings continued through the second 24 hours showing almost the same trend throughout the experimental period.

2. The Ammonium Cultures.

As can be noted from Figure 2, the drop in the nitrogen content proceeded gradually until the 10th day. Then absorption of nitrogen apparently stopped completely, although the nitrogen absorbed was even less than half the amount of nitrogen supplied. From the 10th day onwards, nitrogen was released from the mycelial cells and appeared in the external media. It can also be noted that the total amount of nitrogen absorbed from the ammonium media was much less than that absorbed from the nitrate media. It may be recalled here that *Helminthosporium* spp. have been recorded to be nitrate-utilizing fungi (c.f. LILLY & BARNETT, 1950). However, ROBBINS (1937) in his classification of fungi according to their nitrogen utilization stated that fungi which are able to utilize nitrate nitrogen should be able to use ammonium nitrogen. It may here be added that *Helminthosporium sativum* could flourish on nitrate nitrogen while on ammonium nitrogen it could grow but only to a much less extent. The mycelium in the latter case was weak and slimy compared with a well developed mycelium on nitrate nitrogen.

The inability of the present fungus to flourish on ammonium chloride may be attributed to a gradual drop in the pH value of the culture medium resulting from the preferential absorption of the ammonium ions, and consequently further utilization of ammonium nitrogen was handicapped. This has been met with by several investigators such as HASKINS & WESTON (1950) with *Karlingia rosea*, DARBY & MANDELS (1954) with *Myrothecium verrucaria*, and BARNETT & LILLY (1956) with *Choanephora cucurbitarum*. It is suggested therefore that the attitude of the present fungus towards ammonium nitrogen may change if the pH value of the culture medium is controlled.

The toxicity of the ammonium media as tested by their wilt-inducing capacity to lupin seedlings (Figure 2) remained almost constant for a fairly long period of incubation (from 7th to 22nd day), when dipping lasted for 24 hours only. Later, toxicity increased with longer incubation until the end of the experiment. Filtrate of 34-day-old cultures showed maximum toxicity. The drop in fresh weight of test seedlings was more pronounced when they were kept for 48 hours in the culture filtrates.

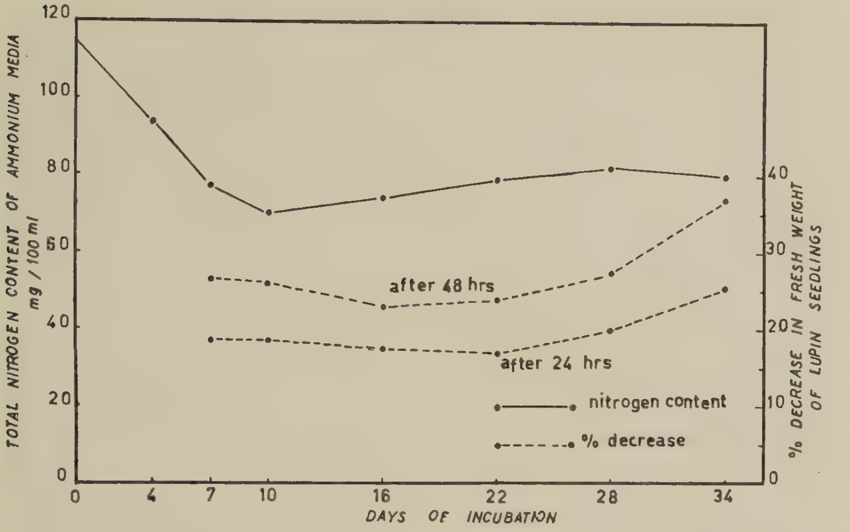

Figure 2. Change in the total nitrogen content of the ammonium media during the incubation period, and the percentage decrease in fresh weight of lupon seedlings, when dipped for 24 and 48 hours, in culture filtrates obtained at corresponding intervals.

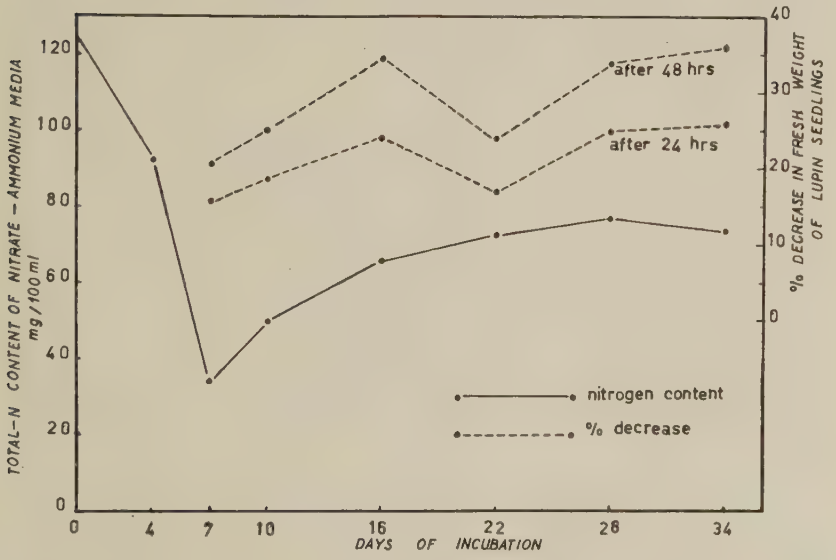

Figure 3. Change in the total nitrogen content of the nitrate-ammonium media during the incubation period, and the percentage decrease in fresh weight of lupin seedlings, when dipped for 24 and 48 hours, in culture filtrates obtained at corresponding intervals.

3. The Nitrate-Ammonium Cultures.

The nitrogen content in these cultures (Figure 3) dropped quite steeply during the first 7 days of incubation until a minimum was reached. It later increased gradually until the 28th day, and then declined. It is interesting to mention that when nitrate nitrogen and ammonium nitrogen were separately estimated, it was found that the fungus utilized only nitrate nitrogen for the first 4 days, and equally nitrate and ammonium nitrogen in the 3 following days.

On the other hand, the toxicity of the culture filtrate, as can also be noted from Figure 3, increased from the 7th to the 16th day, and then dropped again on the 22nd day. Later, it increased gradually until the 34th day when the experiment ended. Two maxima were therefore detected: one on the 16th day, and the other in the vicinity of the 34th day. It may be mentioned in this connection that GÄUMANN (1957) reported according to KLEUPFEL & JUILLARD that the gliotoxin content of culture filtrates of *Aspergillus fumigatus* and *Trichoderma viride* showed two marked quantitative maxima in both stationary and shaken cultures during incubation.

DISCUSSION

The present work was carried out with the aim to trace any correlation that may exist between the nitrogen metabolism of *Helminthosporium sativum*, the causal organism of the leaf-spot disease of barley, and the toxicity of its culture filtrate. PRINGLE & BRAUN (1957) isolated a toxin from the culture filtrate of *Helminthosporium victoriae* and stated that it contained a substance of a peptide nature. It has also been found in other cases, such as tomato wilt caused by *Fusarium* spp. (GÄUMANN & JAAG, 1947), that the product responsible for the disease is a polypeptide. A case of variation of toxicity with change in the composition of the culture medium specially in the nitrogen source has also been reported by HASEN (1926). He found that the metabolic products of *Fusarium vasinfectum* on nitrate were toxic to cotton plants, while filtrates of cultures grown on ammonium salts were not toxic.

On the other hand, recent work on the nitrogen metabolism of fungi, from purely physiological point of view, has revealed that nitrogenous compounds escape from the fungal mycelium into the external medium. NAGUIB (1959) has shown that such extracellular nitrogenous compounds are not of purely autolytic nature, but may be produced while growth is still proceeding. With these findings in mind, it was thought that *Helminthosporium sativum* may release during growth appreciable amounts of nitrogenous compounds that may be, at least partly, responsible for inducing the typical symptoms of the disease when cut shoots of barley are dipped in the filtrate of fungal cultures.

In the present investigation, however, different nitrogen sources were used, namely, sodium nitrate, ammonium chloride, and a mixture of the two with equivalent amounts of nitrogen. In media supplied with nitrate nitrogen, or nitrate plus ammonium nitrogen, appreciable amounts of nitrogen were released out of the mycelial cells, while in media supplied with ammonium nitrogen only, smaller amounts of nitrogen were absorbed and a smaller amount was released into the medium.

When plotting data of nitrogen content of the culture media during the incubation period together with the wilt-inducing capacity of the culture filtrate as represented by percentage decrease in fresh weight of test seedlings, no correlation was obtained in cultures supplied with ammonium nitrogen. Moreover, a higher wilt-inducing capacity was obtained in these cultures than in those supplied with nitrate alone or together with ammonium chloride (except for the 28 day-old cultures), in spite of the fact that the nitrogen released was less in the ammonium than in the other cultures.

In the nitrate and nitrate-ammonium media, only a partial correlation between the accumulation of nitrogen in the culture media and the wilt-inducing capacity of the culture filtrate could be obtained. Maximum wilt from filtrates of nitrate cultures occurred when these were 28 day-old. At that time, the nitrogen content of the culture media was almost at its peak. But the drop in the wilt-inducing capacity of the culture filtrates of the 22 day-old nitrate as well as nitrate-ammonium cultures may show that some other factor was interfering in the process. It may then be assumed that the toxicity of the culture filtrate which is responsible for inducing the symptoms of the disease and which causes wilting to test plants is not due to a single substance of peptide nature, but due to more than one substance; one of these being a nitrogenous product. The observed degree of toxicity would therefore be the result of the interaction between, or the additive action of, these substances.

Finally, it may be mentioned in this connection, that DIMOND (1947) isolated two toxic substances from culture filtrate of *Graphium ulmi*, one is a polysaccharide and the other is an alcohol-soluble component. FELDMAN and coworkers (1950), on the other hand, failed to get a direct correlation between the polysaccharide fraction in the culture filtrate of *Ceratostomella ulmi* and the wilt-inducing capacity of that filtrate.

SUMMARY

When symptoms of the leaf-spot disease of barley caused by *Helminthosporium sativum* could be reproduced by dipping host plants in fungal filtrate, it was thought to trace any correlation that may exist between nitrogenous constituents of the filtrate

and its wilt-inducing capacity to test plants (lupin seedlings). The fungus was grown on a modified Fries' No 3 medium with sodium nitrate or ammonium chloride or an equivalent mixture of both as nitrogen source. The nitrogen content of the culture medium and its wilt-inducing capacity were traced over 34 days. The nitrogen content, after reaching a minimum, was found to rise again; the rise being more rapid and bigger in nitrate and ammonium-nitrate media than in ammonium media. Only a partial apparent correlation could be noted between the trend in the wilt-inducing capacity of filtrates obtained at different periods of incubation, and the trend in the change in their nitrogen content. It is suggested that the toxicity of the culture filtrate may be attributed to more than one substance, one of them being a nitrogenous product (possibly of peptide nature). The observed degree of toxicity would therefore depend on the relative amounts of the nitrogenous product and other substances.

References.

1. Barnett, H. L. & Lilly, V. G. (1956) Factors affecting the production of zygospores by *Choanephora cucurbitarum*. Mycologia, 48, 617—627.
2. Ceponis, M. J. & Friedman, B. A. (1959) Pectolytic enzymes of *Pseudomonas marginalis* and their effects on lettuce. Phytopath. **49, 141—144.**
3. Darby, R. T. & Mandels, G. R. (1954) Inorganic nutrition of *Myrothecium verrucaria*. Mycologia, 46, 276—288.
4. Dimond, A. E. (1947) Symptoms of Duch elm disease reproduced by toxins of *Graphium ulmi*. in culture. (Abst.) Phytopath. **37,** 7.
5. Feldman, A. W., Caroselli, N. E. & Howard, F. L. (1950) Physiology of toxin production by *Ceratostomella ulmi*. Phytopath. **40, 341—354.**
6. Gäumann, E. (1957) Fusaric acid as a wilt toxin. Phytopath. **47, 342—357.**
7. Gäumann, E. & Jaag, O. (1947) Die physiologischen Grundlagen des parasitogenen Welkens. Ber. Schweiz. bot. Ges. **57, 3—34.**
8. Haskins, R. H. & Weston, W. H. (1950) Studies in the lower Chytridiales. 1. Factors affecting pigmentation, growth, and metabolism of a strain of *Karlingia* (*Rhizophlictis*) *rosea*. Amer. J. Bot. **37, 739—750.**
9. Haymaker, H. H. (1928) Relation of toxic excretory products from two strains of *Fusarium lycopersici* to tomato wilt. J. agric. Res. **36, 697—719.**
10. Hockenhull, D. J. D. (1946) Studies in penicillin production by *Penicillium notatum*. 1. A preliminary study of the metabolism of carbon, nitrogen, sulphur, and phosphorus. Biochem. J. **40, 337—343.**
11. v. Hofsten, B. (1956) Growth and metabolism of *Ophiostoma multiannulatum* on different sources of carbon. Physiol. Plantarum, **9, 624—639.**
12. Lilly, V. G. & Barnett, H. L. (1951) Physiology of the Fungi. McGraw-Hill Book Company, Inc. New York. p. 99.
13. Morton, A. G. & Broadbent, D. (1955) The formation of extracellular nitrogen compounds by fungi. J. gen. Microbiol. **12, 248—258.**
14. Naguib, K. (1959) Growth and metabolism of *Aspergillus nidulans* Eidam in surface culture. Can. J. Bot. **37, 353—364.**
15. Pringle, P. B. & Braun, A. C. (1957) The isolation of the toxin of *Helminthosporium victoriae*. Phytopath. **47, 369—371.**
16. Robbins, W. J. (1937) The assimilation by plants of various forms of nitrogen. Amer. J. Bot. **24, 243—250.**
17. Rosen, H. R. (1926) Efforts to determine the means by which the cotton wilt fungus, *Fusarium vasinfectum* produces wilting. J. agric. Res. **33, 1143—1162.**

18. Scandrett, F. J. (1953) A modified all-glass apparatus for the determination of nitrogen by the microKjeldahl method. Analyst, 78, 734—737.
19. Wheeler, H. E. & Luke, H. H. (1954) Studies of a toxic agent produced by *Helminthosporium victoriae*. (Abst.) Phytopath. **44**, 334.
20. White, I. G. & Wolf, F. T. (1954) Toxin production by the oak wilt fungus, *Endoconidiophora fagacearum*. (Abst.) Phytopath. **44**, 334.
21. Zentmyer, G. A. & Horsfall, J. G. (1942) Toxin formation by *Ceratostomella ulmi*. Science, **95**, 512—513.

LIBRI NOVI

A. Frey-Wyssling, *Die pflanzliche Zellwand*, Springer-Verlag, Berlin 1959, 367 p.p. (German) DM 69.60.

This treatise on the plant cell wall is a revised and greatly enlarged edition of a monograph published in 1935; its author is well known for his contributions to modern knowledge of the subject. He divides his survey into three sections, (1) structure and development, (2) chemistry, and (3) biophysics of the cell wall. The first section deals descriptively with the microscopic and submicroscopic structure of the various kinds of cell walls or wall layers, and their possible modes of formation during growth and differentiation, emphasizing and richly illustrating the results of electron microscope study, while drawing on a wide variety of other research as well. The section on chemistry surveys the chemical structure, occurrence and detection of the types of cell wall constituents, but does not attempt very much coverage of or introduction to the extensive chemical literature in this area. The third (biophysics) section discusses fairly extensively the theory and technique of measurement of such properties as X-ray diffraction, birefringence, light absorption,dichroism, density, swelling and tensile strength, showing how they can be used in characterizing the submicroscopic structure, an approach in which Frey-Wyssling has long been active.

Although some literature as late as 1958 is included in the book, the coverage of developments or ideas not even that recent is by no means complete. For example, the polyuronide crosslinkage hypothesis for action of auxin in promoting cell wall growth is not mentioned, and the section on cell wall analysis is oriented largely toward the classical cell wall fractions as distinguished by extraction with different solvents, and important literature both in this area and in cell wall growth is omitted. The possible role of cell wall submicroscopic structure in controlling morphogenesis during growth also receives little attention.

While concerned mostly with higher plant material, there is a good deal of information about cell walls in lower plants scattered through the book, perhaps not as easily available as might be, because of a somewhat spotty index (among matters discussed in the text, which I was surprised to find *not* in the index were *Acetabularia*, root hairs, slime molds, *Acetobacter*, *Oscillatoria*, *Avena*, and coleoptile). About 6 pages are devoted to chitin, and there are shorter treatments of yeast cell wall and cell walls or wall constituents of blue-green and other algae. Bacterial cell walls are also covered, perhaps in rather abbreviated fashion relative to present interest in this subject. There is some, but not extensive, treatment and illustration of types of fungal digestion of cell walls.

The volume certainly affords a valuable source of literature and of illustrative material on cell walls and is a fertile bed of ideas on various aspects of their function and structure.

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HAZEN, E. L. & REED, F. C.: *Laboratory Identification of Pathogenic Fungi simplified*. Pp. 150, illustrated.
C. C. Thomas, Publisher, Springfield, Ill. U.S.A. 1960.

Five years after the first edition of this remarkable text a second edition became necessary which is dedicated to the memory of the late Dr. RHODA W. BENHAM. The objective of the second edition remained the same as that of the first—an aid in the teaching of the essentials in the identification of pathogenic fungi to the beginner. The text deals with the subject matter under three broad headings: (1) Superficial mycoses, (1) Deep-seated mycoses, (3) Contaminants. In an appendix formulae of the most useful culture mediums are presented for the proper isolation and identification of fungi.

The didactic presentation of the subject is most excellent. The discussion of every microorganism is preceded by a "case"bringing the mediums for development of significant characteristic, macroscopically and microscopically. Then, a short, concise description of the clinical appearance of the mycosis follows with the headings: Specimens for examination, Cultural and microscopic characteristics, Pathogenicity for animals. Finally, a full page of illustration brings the features of the giant culture, the microscopical appearance in culture and the fungus' appearance in tissue sections. Drawings are completely eliminated, all the details are presented as photographs and photomicrographs of the highest quality as true documentary evidences. The accompanying text is clear, concise and sufficient. The authors have to be congratulated to their unique didactic achievement. This text is a fine tool for the identification of pathogenic fungi and for the so bothersome contaminants. The availability of this text should be a "must" for every mycological laboratory. Needless to say that the physical appearance of the book in every respect is of Thomas quality. It can be highly recommended.

TIBOR BENEDEK

DEIGHTON, F. C., Studies on Cercospora and allied genera. I. Cercospora species with coloured spores on Phyllanthus (Euphorbiaceae). Mycological Papers, No. 71, Commonwealth Mycological Institute, Kew, Surrey, 23 pp., 11 figs. Price 9 sh.

Ellis, M. B., Clastosporium and some allied Dematiaceae-Phragmosporae. II. Mycological Papers, No. 72. Commonwealth Mycological Institute, Kew, Surrey, 75 pp. 58 figs. Price 25 sh.

The remarkable revival of interest in the imperfect fungi in recent years has been reflected in the numerous papers devoted to them, of which some of the most interesting and valuable have been included in the present series.

Chupp's monograph of *Cercospora* recognized only one species on *Phyllanthus*, a very large, almost entirely tropical genus of the Euphorbiaceae. Deighton recognizes six, describing three as new and redescribing the other three, one under a new name, adding notes on two additional species on related hosts. All are illustrated and a key is given to the six species on *Phyllanthus*.

Ellis discusses a group of widely distributed but scantily collected genera, bringing together scattered references, redefining the genera, making transfers and providing keys to the species of four of them. The genera recognized are *Bactrodesmium*, *Bactrodesmiella* gen. nov., *Brachysporiella*, *Endophragmium*, *Stigmina*, *Clasterosporium* and *Sporidesmium*. Of the 58 species described and illustrated, only 13, including one validated name, bear current names. There are 25 new names or combinations and 20 new species.

Despite the title, it is to be noted that a number of the species could not easily be referred to the genera to which they are assigned by using a key based on Saccardo's system. *Stigmina*, e.g., while predominantly phragmosporous, includes several dictyosporous species. At least one species of *Endophragmium*, as illustrated, would almost certainly, and originally was, referred to the Stilbellaceae. The synonymy of several of the older species is eloquent testimony to the difficulties involved. Nevertheless, while the inadequacies of the Saccardian system are becoming increasingly evident, it has served long and honorably and there is as yet nothing available to take its place. It is earnestly to be hoped that some general revision of the imperfects following current ideas of classification can soon be prepared. The obstacles in the path of such a revision are clearly recognized, and obviously anything of the sort done at the present time would be more than usually tentative. But even so, it would be so useful and the need is so urgent, that a highly provisional revision would be widely welcomed.

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Allergie und Allergische Erkrankungen, edited by E. Rajka, with the collaboration of 21 noted investigators. With 228 illustrations, 52 tables. Vol.II. 1003 pp. Publishing House of the Hungarian

Academy of Sciences. Alkotmány u. 21. Budapest V. (German), 1959.

Vol. I of this handbook, reviewed in this Journal (cf. vol. XII, p. 96), dealt with the general theoretical concepts of Allergology. The present vol. II brings its clinical part. There are chapters on allergic diseases of the respiratory organs, of the gastro-intestinal tract, of the cardio-vascular system, followed by chapters of the allergic diseases of the skin, of the blood and blood forming organs, and of the kidneys. Furthermore, the allergic conditions in tuberculosis and in syphilis are discussed.

The medical mycologist will be mainly interested in the chapter of E. FÉJER on Fungous Allergy. When one would need a classical demonstration, how a one-sided investigation, based on the use of a single tool, may lead to the gravest clinical and etiologic errors, this chapter would provide all the evidences. Allergy and allergic skin reactions are the only principles, by means of which the author is able to look at dermatoses. Results of investigations of the last three decades obtained by the classical and concurring methods of bacteriology and histopathology do not enter the picture at all. A fungus culture is equated with fungus disease and adding a positive skin test, we have an allergic fungus condition. The center of all troubles is the interdigital and nail "mycoses" as a constant, feeding source of allergens. That in "interdigital mycosis" the primary lesion is a tiny blister and/or pustule which may or may not be sterile for the specific causative organism is nowhere mentioned. It is apparently unknown to the author that up to now there are only three cases of known primary onychomycosis, those described by STÜHMER. Classical forms of seborrheic dermatitis, nummular eczema and even neurodermatitis are of mycotic origin since they show positive allergy to this or that fungus extract. The dermatological nomenclature is most confusing. There are "dysidrosiform epidermophytids", "dysidrosiform eczemas", epidermophytids which look identical with the "dysidrosis" of TILBURY FOX, which in turn, as we learn, is identical with the pompholyx of HUTCHINSON . . . RAVAUT and RABEAU'S "levurids" are revived as classical examples and manifestations of allergy to yeast-like fungi, although these levurides are classical examples, histopathologically and bacteriologically, of seborrheic dermatitis, an endoparasitic skin condition.

Almost the whole dermatology is included in the fungus allergic manifestations, and explained away in allergologic terms with complete disregard of classical clinical dermatology, histopathology and bacteriology. This trend of allergologic research is discrediting the seriousness of the method and its otherwise well established results. Skin testing and an allergic reaction might be a clinical help but not a supreme judge in establishing an etiologic diagnosis. In this respect we face the same facts as with the results of serology. We have at present about forty different causes of a biologically unspecific serologic reaction for syphilis!!

In FEJÉR'S chapter we have the final impression after its careful scrutiny that dermatology and dermatomycology have to be saved of allergology. As to the positive fungus cultures one is reminded of the old adage:" I saw a rabbit climb on a tree, if you don't believe it, I'll show you the tree".

TIBOR BENEDEK

CLARKE, G.H.V.: *Skin Diseases in the African*
Pp. VIII & 169, Demy 4to. With 261 illustrations in the text, 1 colored plate and an extensive bibliography. Price: £ 4 4s or $ 11.80. H. K. Lewis & Co. Ltd. London, 136 Gower Street, W.C.1. 1959.

This neatly presented volume gives a good opportunity to medical mycologists to enlarge their horizon. This volume is devoted exclusively to dermatology in the Negro based on the author's own experience in West Africa, the homeland of the Negro race. Diseases more or less peculiar to the African are treated more exhaustively than those diseases common also in the white race. As one can expect Nigeria and more particularly, Southern Nigeria with its humid, tropical climate is a hot bed for fungus diseases. Among the dermatological cases coming to the Lagos, Nigeria clinic fungus diseases (all forms) with 20.6 % take the second place in frequency! Tinea capitis is common due to *Microsporon audouinii*. Other species frequently encountered are *T. soudanense*, *T. ferrugineum*, *T. violaceum*. *M. felineum* is apparently absent. Favus (*A. schoenleinii*) is common. In tinea corporis *T. rubrum* is leading with 60 %. *T. ferrugineum* and *T. soudanense* attack the glabrous skin, too. Dealing with the superficial fungus infections there is a misleading caption. In fig. 233 it is said "'Pinta' from Tinea flava achromia parasitaria (blood serology negative)". Since 1938 we know that "pinta" is not a fungus disease (although mycologists excelled in discovering a host of the most different fungi in the scaly lesions of "pinta") but a spirochaetosis. Furthermore "blood serology" cannot be negative or positive, for that matter, the author certainly means the "serologic test" of the blood. Interesting is the absence of the deep seated mycoses. North American, South American and European blastomycoses were not reported. Coccidioidomycosis is absent. Chromoblastomycosis, histoplasmosis were observed. The illustrative material is excellent, the most extensive bibliography is useful. The production of the book is outstanding. It can be highly recommended.

TIBOR BENEDEK

HUNTER, G. H., FRYE, W. W. and SWARTZWELDER, J. C.: A Manual of Tropical Medicine. Third edition. Illustrations: 323, 8 in color, 892 p. W. B. Saunders Co. Philadelphia, Pa. Price: $ 15.—.

Tucked away in larger volumes of medical treatise of general scope there often are short chapters on medical mycology to round out the whole picture. This is the case in this volume of a well known, much used Manual. The chapter on mycotic diseases is written by the distinguished mycologist, NORMAN F. CONANT and encompasses altogether 40 pages. It succinctly covers the whole range of superficial and deep seated (systemic) mycoses.

Medical mycology is a borderline science, i.e. at one end as far as superficial mycoses are concerned, deeply dips in clinical dermatology; at the other end, i.e. concerning the deep seated (systemic) mycoses, it reaches far into the field of internal medicine proper. For that reason, "pure" mycologists have a difficult time and walk on an extremely thin ice at the moment, when the etiologic and pathogenetic significance of fungal organisms come up to be evaluated. Due to these facts, one has to scrutinize such short treatise from both the mycological and from the clinical angle. As far as the mycological facts are concerned, CONANT's presentation is correct. Concerning the clinical evaluation of the fungal organisms it is a chain of unqualified errors. In the clinical part CONANT relied on a wholly misinformed dermatological advice, bringing the illustrative material of NOOJIN and CALLAWAY of Duke University. "Dermatophytosis", "Onychomycosis", "Otomycosis" are treated in etiology and pathogenesis as it was known thirty years ago. The research of the last three decades went by completely unnoticed.

CONANT is not only mycologist but also a noted bacteriologist and thus, he has all the qualifications to control that "dermatophytosis", "onychomycosis" are etiologically and pathogenetically not of fungus origin, but bacterial-endoparasitic. What is called "otomycosis" is again not a fungus disease of the external ear canal; fungi (*Penicillium, Mucor, Aspergillus*) vegetate there in detritus masses of the inflamed skin of an entirely different etiologic origine. Fungi do not play any part in it. Due to these grave errors the part of superficial mycoses has no value at all. The deep seated mycoses are presented in the usual fashion. There are no etiologic or pathogenetic errors, since the diagnosis basically rests on the histopathological findings.

The volume as a whole excellently presents the material on tropical diseases with well selected illustrative and graphic material. The physical appearance of the volume is excellent.

TIBOR BENEDEK

A. CHAVES BATISTA; *Monografia dos fungos Micropeltaceae*. Publicação No. 56, Instituto de Micologia, Universidade do Recife. One vol. Pp. 519. with 228 fig. Recife 1959.

The family, Microthyriaceae (Hemisphaeriaceae) order Microthyriales (Hemisphaeriales) are tropical, mostly foliar Ascomycetes, characterized by small (about 1 mm) superficial thyriothecia, as a rule meandriform, without internal and free mycelium.

It is quite a critical group of fungi, whose species are encompassed in four subfamilies, including about 45 genera, fifteen being new for science. Many new species and new combinations are proposed, in part, after direct revision of the specimens, or accepted on the basis of a critical revision of the previous descriptions. Almost all the species studied on the real material are illustrated.

This monograph is based on classical morphological characteristics: shape of the ascostromata; presence or absence of the ostiole and type of dehiscence; presence and number of septa of the ascospores; presence or absence of paraphysis. If present, the pycnidiospores are described, but the species not named.

This massive and well illustrated monograph is a thorough revision of actual knowledge on this little known family of fungi, and the Author and his staff is to be congratulated for the work.

Professional mycologists, for whom this publication is intended, can judge its merits; after continued use, they may be in a condition to decide the usefulness of the application of SACCARDO's strict criteria for this group of fungi. That it will be used, and, despite possible shortcomings, it will become a standard, is beyond question.

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HEUNERT, H. H.: *Praxis der Mikrophotographie*. 2. Auflage. Mit 80 Abbildungen in 120 Einzeldarstellungen, VII, 96 Seiten, Gr. 8. Springer-Verlag, Berlin, 1959, Preis: D.M. 19.80. (German).

Although we have a number of outstanding texts on photomicrography in English, we feel that it is always useful to look into what the other fellow does and how he does it. Under four headings this text presents (1) equipment for photomicrography, (2) methods of microscopical investigations (bright and dark field,' phase contrast, polarized light, fluorescence), (3) technique of exposure, (4) advice from the practice for the practice. The illustrative material for equipment and for test examples in animal and plant tissues is numerous and excellent. The production of the book is in every respect the best. This reviewer repeatedly had the opportunity to underscore the importance of photomicrography in mycological work of any and every description. It is always documentary. This small, excellent volume can be highly recommended.

TIBOR BENEDEK

GRAY, W. D.: The Relation of Fungi to Human Affairs. Profusely illustrated. 510 p. Holt, Rinehart and Winston, Inc. New York, N.Y. Price $ 8.50, 1959.

This volume presents the subject of mycology in a most unusual way. In twenty-five chapters it deals with the beneficial activities of Fungi (Part I) and then, it discusses their harmful activities (Part II). In this comprehensive and penetrating text, the author considers both aspects, describing in detail not only the harmful activities, but also the many current uses and vast potential of fungi as controlled agents working for man. Throughout the text the author emphasizes fungus physiology and function, rather than fungus morphology. There are chapters on destruction of organic waste, on industrial mycology, on alcoholic fermentation, on the synthetic activities of fungi (synthesis of gallic, citric, gluconic acids, etc.), on fat production, etc. The chapters on harmful activities deal with plant diseases, destruction of timber and timber products, tropical deterioration of materials, food spoilage, etc. Finally a short chapter deals with medical mycology.

The volume is excellently written in a most interesting context with human affaires. The illustrations, the graphs are well selected and contribute a great deal to the understanding of the text. Every chapter has its own well selected bibliography. A good index enhances the usefulness of the volume. This volume presenting mycology in the most interesting, dynamic way can be warmly recommended for the library of every mycologist.

TIBOR BENEDEK

CORRECTION

In vol. XII/4, page **361** in the review of SCHWARTZ et al.: Occupational Diseases of the Skin, the following sentence: "The causative organism of chromoblastomycosis does not grow by budding in tissue and has a dark, brown color in culture", was used by oversight in a negative sense. This sentence is herewith considered eliminated from the context of the review.

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DESCRIPTIONS OF SOME FUNGI ASSOCIATED WITH FOREST TREE DECAY IN COLORADO

Hazel H. McKay[1]) & Paul L. Lentz[2])

(24.III.1960)

Introduction

For several years, R. W. Davidson and associates have studied timber decays and collected wood-decaying fungi in the Rocky Mountains of Colorado. The majority of their collections were made in the moist subalpine zone at the 8,500-to 11,000-foot elevation and the host trees included Engelmann spruce (*Picea engelmannii* Parry), subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), aspen (*Populus tremuloides* Michx.), and relatively few lodgepole pine (*Pinus contorta* Dougl.). Many collections were of relatively inconspicuous but destructive Basidiomycetes, including species in the Tremellales and Thelephoraceae. Decays caused by four species were discussed recently by Davidson & Hinds (11); their discussion is supplemented herein by data on basidiocarp and cultural characteristics.

Peniophora rufa (Fr.) Boidin is related to and grows in the same environment as one of the species discussed by Davidson & Hinds; therefore it is included among the species to be characterized even though it was not considered by them.

HELICOBASIDIUM CORTICIOIDES

(Figs. 1, A; 2, A—C; 3, A; 4)

Helicobasidium corticioides Bandoni, Mycologia 47: 918. 1955.

Basidiocarp Description

Macroscopic Characteristics: Basidiocarp (Fig, 1, A) forming a thin, adherent, arid, whitish felt on coniferous wood.

Microscopic Characteristics: Structure exactly as described by Bandoni (1), with hyphae (Fig. 4, A) septate, lacking clamp

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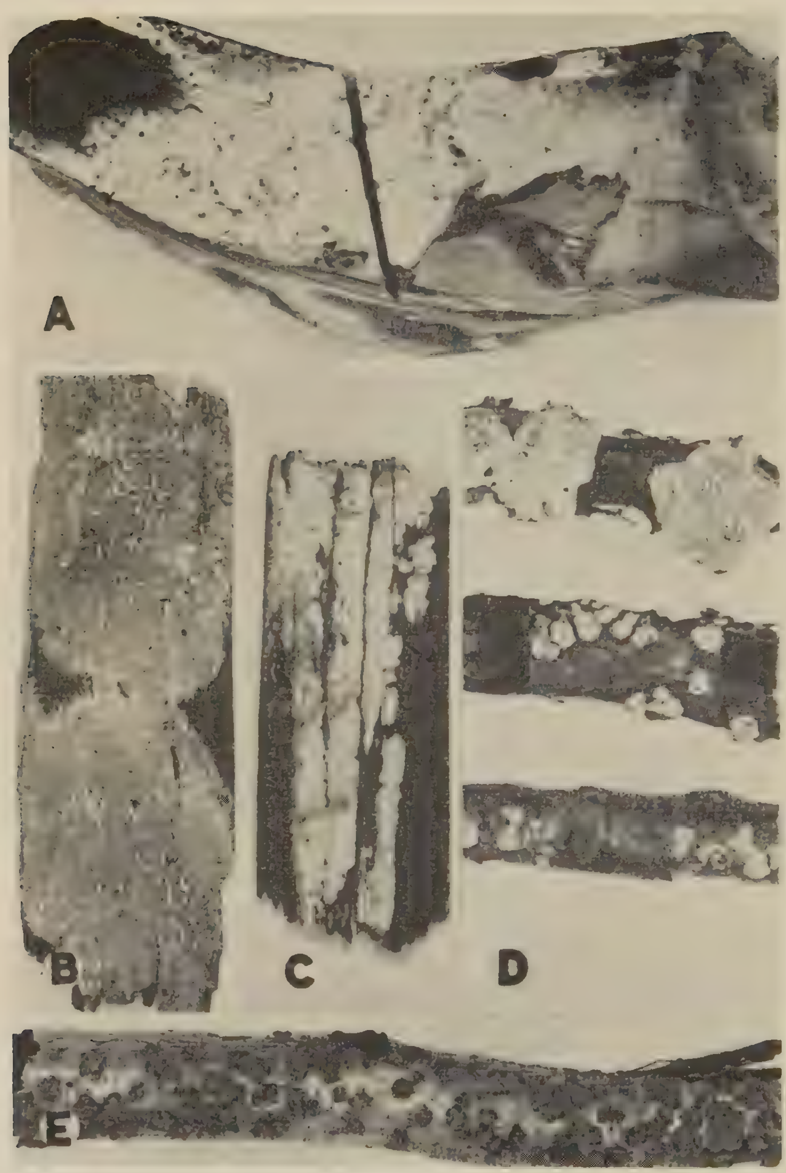

Fig. 1. Habit photographs showing appearance of herbarium specimens. A, *Helicobasidium corticioides*; B, *Corticium radiosum*; C, *Peniophora luna*; D, *P. polygonia*; E, *P. rufa*. All × 1.

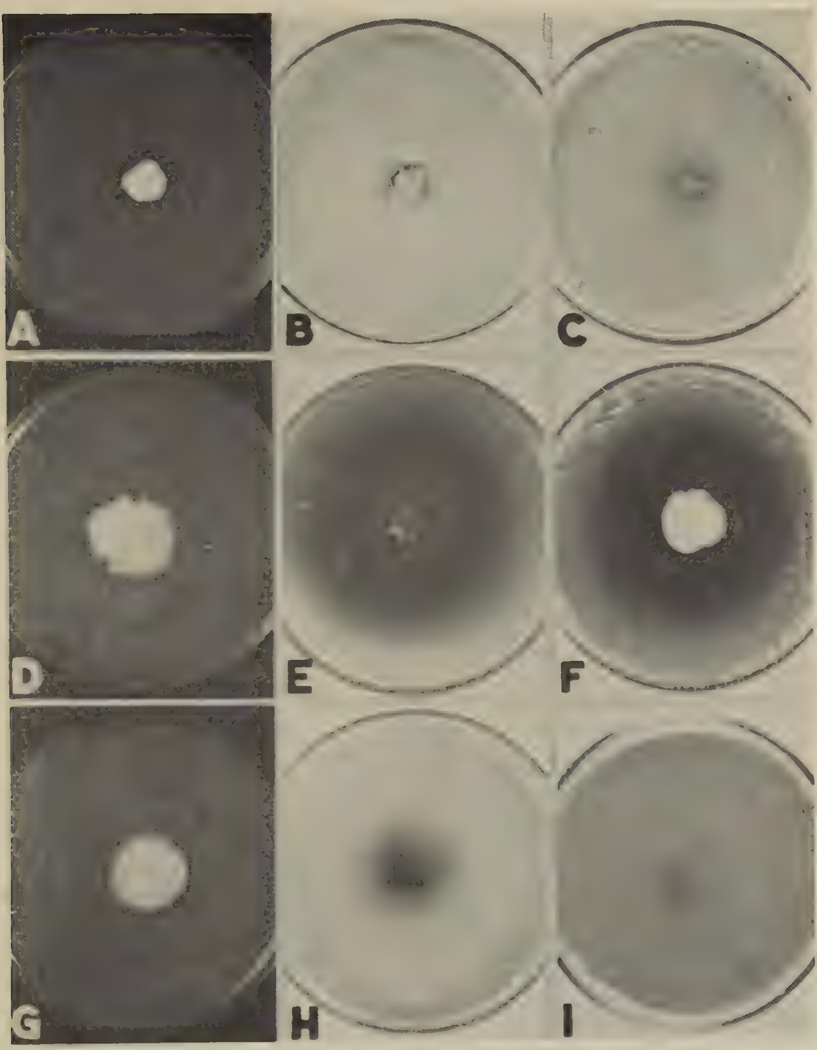

Fig. 2. Cultures photographed at 14 days' growth on: A, D, G, diamalt agar; B, E, H, gallic acid agar; C, F, I, tannic acid agar. A—C, *Helicobasidium corticioides*; D—F, *Corticium radiosum*; G—I, *Peniophora luna*. All approximately × 1/2.

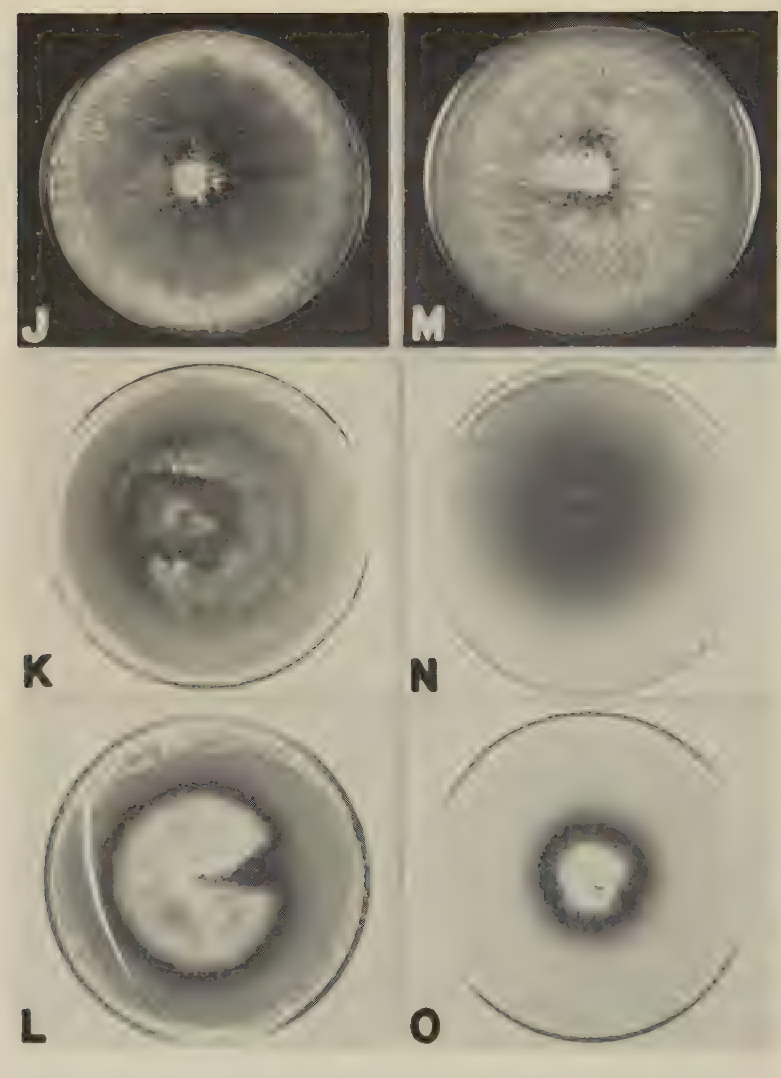

Fig. 2 bis. Cultures photographed at 14 days' growth on: J, M, diamalt agar; K, N, gallic acid agar; L, O, tannic acid agar. J—L, *Peniophora polygonia*; M—O, *P. rufa*. All approximately × 1/2.

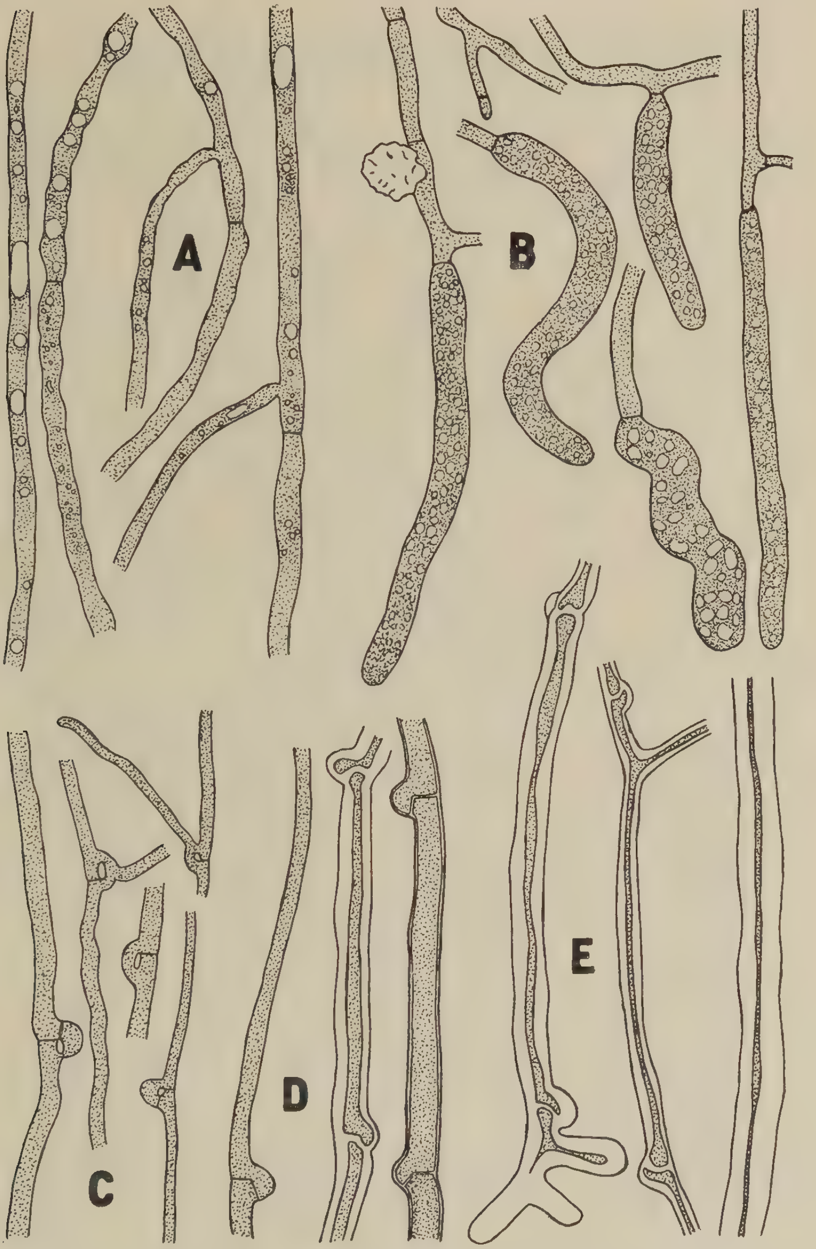

Fig. 3. Camera lucida drawings showing characteristic anatomy of hyphal elements: A, *Helicobasidium corticioides* hyphae at 14 days; B, *Corticium radiosum* hyphae and gloeocystidium-like hyphal segments at 14 days; C, *Peniophora luna* hyphae with loop-like clamp connections at 14 days; D, *P. polygonia* hyphae showing the appearance of two with thin walls at 14 days and one with thick walls at approximately 21 days; E, *P. rufa* hyphae with thick walls at 14 days. All × 1000.

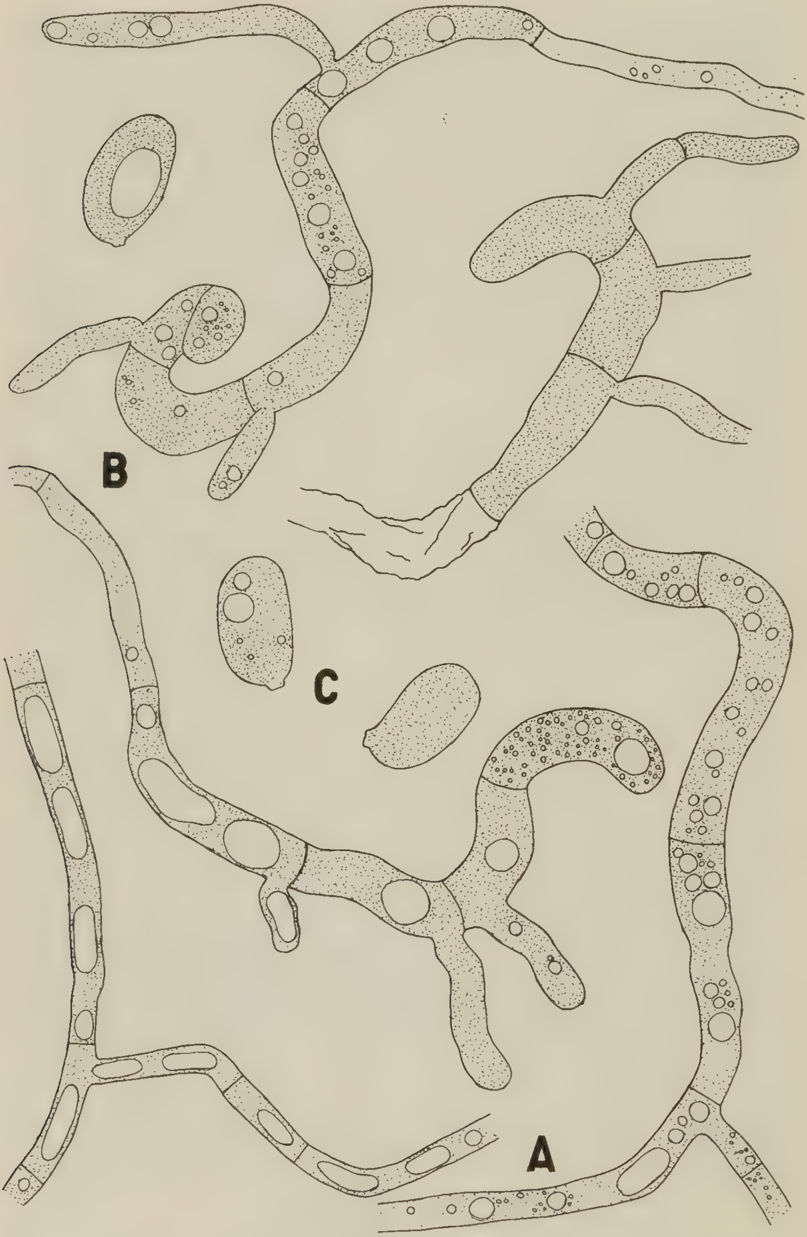

Fig. 4. Camera lucida drawings showing basidiocarp elements of *Helicobasidium corticioides*: A, hyphae; B, basidia; C, spores. All × 1000.

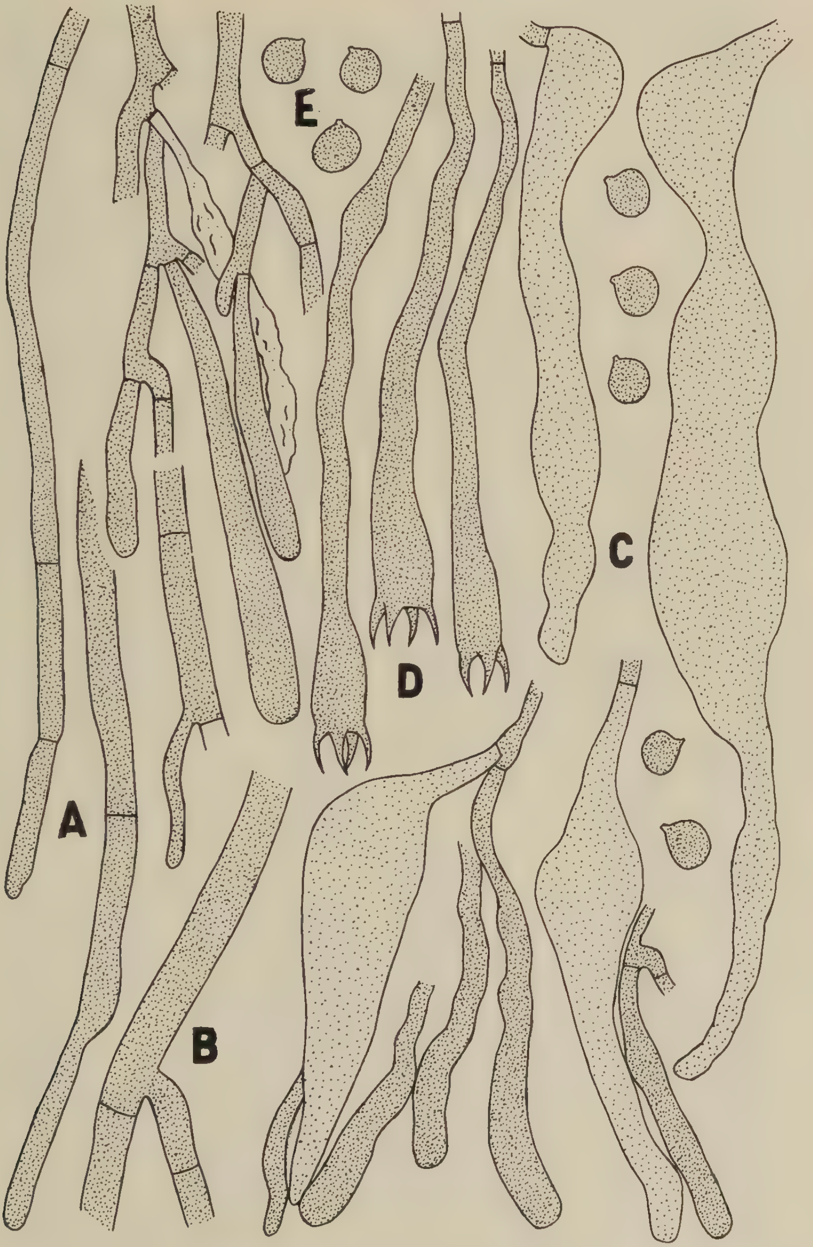

Fig. 5. Camera lucida drawings showing basidiocarp elements of *Corticium radiosum*: A, hyphae from the basidiocarp margin showing narrow apices; B, context hyphae; C, gloeocystidia; D, basidia; E, spores. All × 1000.

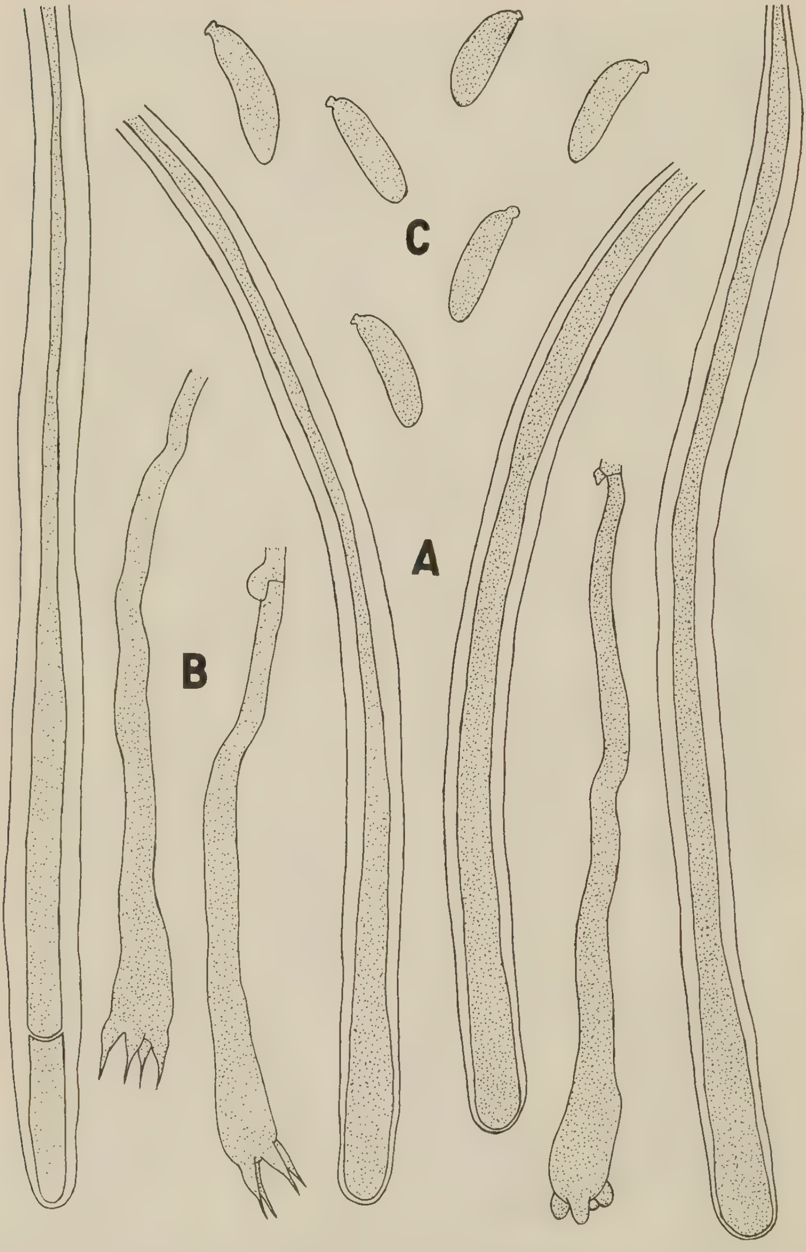

Fig. 6. Camera lucida drawings showing basidiocarp elements of *Peniophora luna*: A, cystidia; B, basidia; C, spores. All × 1000.

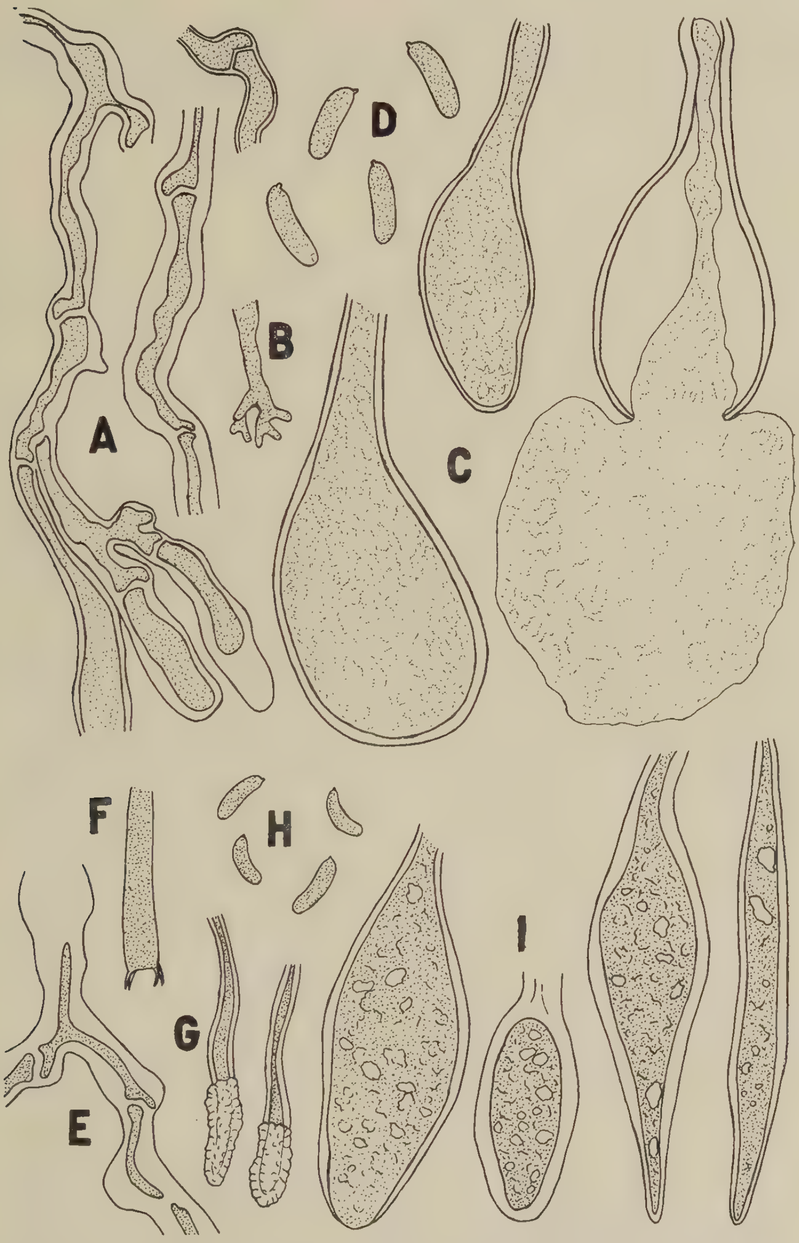

Fig. 7. Camera lucida drawings showing basidiocarp elements. A—D, *Peniophora polygonia*: A, thin- and thick-walled hyphae with clamp connections; B, dendrophysis; C, gloeocystidia, with contents exuding from one; D, spores. E—I, *P. rufa*: E, thick-walled hypha showing clamp connections; F, basidium; G, cystidia; H, spores; I, gloeocystidia. All × 1000.

connections, approximately 3—7.5 μ in diameter, containing numerous oily globules; basidia (Fig. 4, B) usually 4-celled, variously arched or somewhat coiled; spores (Fig. 4, C) hyaline, non-amyloid, 16.5—18 × 8—9 μ (14 — 22.5 × 6.5 — 12.5 μ according to BANDONI).

Culture Description

Key Pattern: A-O-V-10[3]).

Growth Characteristics: Growth on 2.5 % Fleischmann's diamalt agar at 18° C very slow, with mat varying from a trace to a diameter of 12 mm in diameter at 14 days (Fig. 2, A), a trace to 36 mm at six weeks, and 22—54 mm at 12 weeks, thickened and woolly over the inoculum block at 14 days, elsewhere fragile and adherent, within 12 weeks becoming much thickened at the center and forming a very thick loose-woolly growth that becomes gradually thinner toward the margin, with the marginal region thin, downy, translucent, and the edge fimbriate and appressed; upper surface of mat entirely white; the underside of mat at first white, but showing a delicate tinge of "salmon color"[4]) within 12 weeks; culture odorless; oxidase reaction negative on gallic acid and tannic acid media (Fig. 2, B, C), mycelium failing to grow on either medium or rarely exhibiting a trace of growth on gallic acid medium.

In test-tube cultures at 28 days the mat covering slant and upper portion of diamalt agar cylinder, forming a deep and loose-woolly mound of growth over and around the inoculum block, gradually diminishing in thickness toward the margin; the mat white; agar under center of mat exhibiting very faint "tawny" discoloration.

Microscopic Characteristics: All hyphae (Fig. 3, A) staining with phloxine, 0.5—1 (—3) μ in diameter, septate, lacking clamp connections, infrequently branching, the older hyphae in the center of the mat occasionally developing torulose swellings.

Temperature Relations: The optimum growth temperature was 16° C, the minimum 8—10°, and the maximum approximately 26° (Table I). After 21 days at 28°, cultures grew when returned to 16° for 14 days. After 21 days at 30°, cultures failed to grow when returned to 16°.

Cultures Studied: Isolates studied as the basis for describing cultural characteristics were obtained from germinated spores of basidiocarps collected in Colorado, as follows: F. P. 100352-Sp[5]) and

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[3]) The methods used in studying the cultures, terminology, arrangement of the descriptions, and explanation of the key pattern are those used in previous studies in this laboratory (10). Cultures were incubated at 18° or 25° C. The test for oxidase reaction follows the methods described by DAVIDSON et al. (9).

[4]) Colors given in quotation marks are from RIDGWAY (24).

[5]) The prefix F. P. is a designation applied to cultures in the permanent series of the Forest Disease Laboratory. The suffix -Sp indicates that the culture was originally obtained from germinated spores from a basidiocarp. Thesuffix -R, cited for some cultures, indicates that the culture was originally isolated from a rot of the host tree.

TABLE I.

Average mat diameters of fungi grown at constant temperature (centigrade) for 10 days (P. polygonia and P. rufa) or 21 days (H. corticioides, C. radiosum, and P. luna).

Fungus Name	8°	10°	12°	14°	16°	18°	20°	22°	24°	26°	28°	30°	32°	34°
Helico-basidium corticioides	*tr	tr	** 15.2	13.5	21.7	17	13.9	14	10	tr	0	0		
Corticium radiosum	11.8	13	15.2	21.8	22.5	29.8	38.2	34.1	34.8	27.5	13.8	10	tr	0
Peniophora luna	11	11.5	15.3	18.8	27.4	31	32.1	38.6	33.8	30	16	tr	0	0
Peniophora polygonia	10	16.3	15.3	27.7	40	49.1	70	75.2	80.6	82	71	24.7	16.7	0
Peniophora rufa	tr	tr	13.2	18.5	24.7	41.6	47.6	62.4	69.8	66.2	60.3	53.3	27	tr

* tr = trace of growth or mat less than 10 mm in diameter.

** Measurements of mat diameters in millimeters represent averages of all cultures of a species grown in triplicate.

F. P. 100364-Sp on *Abies lasiocarpa*; F. P. 100295-Sp and F. P. 100355-Sp on *Picea engelmannii*; F. P. 100348-Sp on *Pinus contorta*; F. P. 105168-Sp on coniferous log. Several of the cultures were obtained from basidiocarps listed by BANDONI in his original description. These include F. P. 100348 (basidiocarps cited by BANDONI as Colo. 766), F. P. 100352 (Colo. 784) F. P. 100355 (Colo. 791), and F. P. 100364 (Colo. 833).

CORTICIUM RADIOSUM

(Figs. 1, B; 2, D-F; 3, B; 5)

Corticium radiosum (FR. ex FR.) Fr., Epicr. 560. 1838; Hym. Eur. 649 1874.
Thelephora radiosa FR. ex FR., Elench. 1: 206. 1828; Obs. Myc. 2: 277. 1818.
Thelephora alutacea FR., Syst. Myc. 1: 451. 1821, ut syn. *T. Himantia laevis* SCHRAD., Spic. Fl. Germ. 187. 1794.
Athelia ochracea PERS., Myc. Eur. 1: 84. 1822.
Thelephora citrina PERS., Myc. Eur. 1: 136. 1822.
Corticium alutaceum (SCHRAD.) BRES., I. R. Accad. Agiati Atti, Ser. III, 3: 110. 1897.
Gloeocystidium alutaceum (SCHRAD.) BOURD & GALZ., Bull. Soc. Myc. France 28: 367. 1913.
Gloeocystidium citrinum (PERS.) LUNDELL, in LUNDELL & NANN-

FELDT, Fungi Exs. Suec. Praesert. Upsal., Fasc. XV — XVI, no. 743. 1939.

Gloeocystidiellum alutaceum (SCHRAD.) BOIDIN, C. R. Acad. Sci., Paris 233: 825. 1951, lacking citations.

Gloeocystidiellum citrinum (PERS.) DONK, Fungus 26: 9. 1956.

Gloeocystidiellum radiosum (FR. ex FR.) BOIDIN, Publ. Mus. Nat. Hist. Nat. no. 17: 122. 1957.

Basidiocarp Description.

Macroscopic Characteristics: Basidiocarp (Fig. 1, B) forming a resupinate, smooth, "cream buff" to "warm buff" coating on the substratum; the hymenial surface sometimes cracking into small areas with the white context visible through the cracks; margin radiating, fibrillose, white, usually not more than 1 mm broad in mature specimens but sometimes with delicate radiating strands extending several mm beyond the hymenial margin.

Microscopic Characteristics: Hyphae (Fig. 5, A, B) colorless, septate, lacking clamp connections, 2.5—3 μ in diameter in the subhymenium, up to 6 μ broad in the context, with many hyphae of the advancing margin having narrowed apical portions (Fig. 5, A); gloeocystidia (Fig. 5, C) having various forms, usually elongated and often basally ventricose, approximately 45—135 × 11—18 μ; basidia (Fig. 5, D) 4-sterigmate, approximately 75—87 × 6—8 μ; spores (Fig. 5, E) smooth, hyaline, weakly amyloid, 5.5—6.5 × 4.5—6 μ.

Specimens Examined: Specimens of the various species under discussion were selected from the National Fungus Collections for study, and designated authorities, collectors, and localities are cited as they appear on the herbarium packets. It does not seem necessary to cite specimens of the other species examined, for the concepts presented as the result of studying those specimens are the ones generally accepted by mycologists.

However, various mycologists have presented differing concepts of *C. radiosum*. As a basis for discussing these concepts, the following specimens were examined (1) *C. radiosum* FR., Cameron Pass, Colorado, F. P. 100327, det. P. L. LENTZ; (2) *C. radiosum* FR., Shelburne, Vermont, coll. G. ENO, det. H. S. JACKSON; (3) *C. radiosum* FR., Bethel, Vermont, coll. P. SPAULDING (Nov. 20, 1912), det. E. A. BURT & J. R. WEIR (4) *C. Gloeocystidium radiosum* FR., Coolin, Idaho, coll. J. R. WEIR (Aug. 13, 1920), det. D. P. ROGERS; (5) *Gloeocystidium alutaceum* (SCHRAD.) BOURD., Ruzbachtal in Stubai, Tirol, coll. V. LITSCHAUER, Weese Eumycetes Selecti Exs. 59; (6) *G. alutaceum* (SCHRAD.) BOURD & GALZ., in valle rivi Liscenka prope vecum Trebusany (Aug. 1936), coll. and det. A. PILÁT.

Culture Description

Key Pattern: A-P-S-10-14-16; B-P-V-10-16.

Growth Characteristics: Growth on 2.5 % diamalt agar at

25° C slow or very slow, with mat varying from a trace to a diameter of 20 mm at seven days, 16—37 mm at 14 days (Fig. 2, D), 31—44 mm at 21 days, thickened and floccose over the inoculum block, elsewhere thin and flat-downy, with the margin raised and even, the mat sometimes having indistinct wavy lines of zonate growth and also with lines of delicately granular growth radiating from the center and spreading to form fan-like marginal lobes, the radiating lines often beset with scattered small dense flecks of raised growth; upper surface of mat at first white, then "pale buff" to "ochraceous-buff" in the center, with the marginal area translucent and revealing the color of the agar when examined from above; the underside of the mat white, or more often "ochraceous-tawny" to "cinnamon-brown" in the center; culture odorless; the oxidase reaction strongly positive on gallic acid and tannic acid media (Fig. 2, E, F) with the mycelium either failing to grow or exhibiting a trace of growth on gallic acid medium but with growth on tannic acid medium similar in extent and appearance to that on diamalt agar.

In test-tube cultures on diamalt agar at 28 days the mat remaining small, covering the inoculum block as a downy to felty growth, then spreading outward as a thin, translucent marginal growth of silky strands, with the margin raised and having an irregularly wavy to lobed configuration with an even edge; the mat white to "light buff"; the agar not discolored; culture odorless.

Microscopic Characteristics: All hyphae (Fig. 3, B) staining with phloxine, 1—3.5 μ in diameter, septate, lacking clamp connections, the hyphae in the center of the mat often profusely- and short-branched, twisted and contorted; occasional hyphae encrusted by a few large usually octahedral crystals at 14 days, with encrusted hyphae numerous at 21 days; gloeocystidium-like hyphal segments (Fig. 3, B) abundant in all cultures, 32—90 × 3.5—4.5 μ, with walls slightly thickened, hyaline or pale brown, and with contents dense and becoming deeply stained by phloxine.

Temperature Relations: The optimum growth temperature was 20° C, the minimum less than 8°, and the maximum 32° (Table I). Cultures were killed by incubation at 34° for 21 days.

Cultures Studied: Cultures from Colorado studied as the basis for describing cultural characteristics include: F. P. 100316-Sp, F. P. 100324-Sp, F. P. 100327-Sp ,F. P. 105154-Sp, and Colo. 52-863[6]), all on *Abies lasiocarpa*; Colo. 57—13, Colo. 57—45, Colo. 57—59, Colo. 57—120, F. P. 100308-Sp, F. P. 100310-Sp, and F. P. 104484-Sp, all on *Picea engelmannii*; F. P. 100294-Sp, F. P. 100313-Sp, and F. P. 100335-Sp, all on coniferous logs.

Remarks: Cultures of *C. radiosum* are recognizable by slow growth, flat-downy mat with fan-shaped spreading marginal growth, very dark oxidase reaction, absence of clamp connections, and curved-cylindrical gloeocystidium-like hyphal segments.

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[6]) The prefix Colo. refers to cultures collected in Colorado and not yet assigned a permanent place in the F. P. series.

Discussion

The basidiocarps of *C. radiosum* differ in at least two important aspects from the recent description by CUNNINGHAM (8) and in at least one respect from that by KÄÄRIK & RENNERFELT (17). In addition, the cultural characteristics reported by the latter are not like those observed during the present study. CUNNINGHAM found clamp connections on the hyphae of the basidiocarps which he examined, and the illustration in his Text Fig. 10 shows that these were relatively abundant. Moreover, the spores of his specimens were reported as measuring 8—11 × 7—8 μ. KÄÄRIK &RENNERFELT evidently repeated, at least in part, the description of basidiocarps given originally by BOURDOT & GALZIN (5); thus it is difficult to determine how closely their basidiocarps resemble those from Colorado. However, they reported the formation of clamp connections in culture and also other cultural characteristics that deviate from those of the Colorado collections. KÄÄRIK & RENNERFELT listed their collections as *C. alutaceum* BRES., with *C. radiosum* given as a synonym. It is interesting, and perhaps pertinent in relation to the fungus of KÄÄRIK & RENNERFELT, to note that LYMAN (22) previously described *C. alutaceum* as having clamp connections. In cultures of his fungus, LYMAN observed the formation of small, bulbous sclerotium-like groups of cells which he called "bulbils". BIGGS (2) commented that LYMAN'S fungus almost certainly belongs in *C. coronilla* HÖHN & LITSCH., Group IIIc, as described in her culture studies. ROGERS (25) included both *C. coronilla* and *C. alutaceum* sensu LYMAN as synonyms of *Trechispora brinkmanni* (BRES.) ROGERS & JACKS.

In the present study, no clamp connections were found either in basidiocarps from North America and Europe or in cultures obtained from Colorado. Thus clamp connections apparently are not formed by *C. radiosum*. This conclusion coincides with that expressed by JACKSON (16) and BOIDIN (3). The spore measurements cited by CUNNINGHAM are markedly greater than those found during the present investigation. BURT (7) reported the spores as subglobose and 4.5—7 μ in diameter or 6 × 4.5 —5 μ. BOURDOT & GALZIN (5) found them to measure 4—7 × 4—6 μ. Thus the characteristics described by CUNNINGHAM are not those of *C. radiosum* as understood by various other mycologists.

BURT'S description of *C. radiosum* does not include mention of gloeocystidia but only of vesicular bodies. This failure by BURT to report the true nature of gloeocystidia was previously discussed by ROGERS & JACKSON (26). However, it is interesting that a slide in the National Fungus Collections from BURT bears his notation, "Gloeocystidia destroyed by KHO" (sic). *C. radiosum* belongs among a group of species which DONK (12) named *Gloeocystidiellum*. The distinguishing characteristics of that genus include sulfo-aldehyde-positive gloeocystidia and amyloid spores.

PENIOPHORA LUNA

(Figs. 1, C; 2, G-I; 3, C; 6)

Peniophora luna ROMELL ex ROGERS & JACKS., Farlowia 1: 320. 1943.

According to ROGERS & JACKSON (26), *Peniophora odorata* sensu BURT (6) and BOURDOT & GALZIN (5) and *Stereum odoratum*, *Xerocarpus odoratus*, and *Phanerochaete odorata*, all sensu KARSTEN (18, 19, 20), are synonymous with *P. luna*.

Basidiocarp Description

Macroscopic Characteristics: Basidiocarp (Fig. 1, C) forming a tough-felted "pale buff" cushion-like pad on the substratum, up to 1 mm thick, with marginal growth restricted except by confluence with adjacent basidiocarps; margin abrupt and concolorous with the hymenial surface.

Microscopic Characteristics: Hyphae septate, with clamp connections; cystidia cylindrical, with rounded apices, non-septate or with occasional very thin septa, not encrusted, up to 300 μ long and 10 μ broad (80—150 × 6—9 μ according to BURT (6)), many cystidia (Fig. 6, A) long-exserted; basidia (Fig. 6, B) 4-sterigmate, with sterigmata originating as prominent knobs and then with a very slender spicule developing from the apex of each knob, the basidia up to 95 μ long and 8 μ broad (40—85 × 7.5 —9.5 μ according to ROGERS & JACKSON (26)), occasionally, or perhaps often, subtended by a clamp connection that may appear only as a clamp wall remnant; spores (Fig. 6, C) cylindrical to subfusiform, each with a broad bottle-mouth-shaped apiculus, hyaline, non-amyloid, 14—19.5 × 4—5 μ(12—15 × 4—6 μ according to BURT (6) or 15—18 × 4.5 —7 μ according to ROGERS & JACKSON (26))

Culture Description

Key Pattern: A-O-V-1-10; B-O-S-1-10-16.

Growth Characteristics: Growth on 2.5 % diamalt agar at 25° C slow or very slow, with mat varying from a trace to a diameter of 18 mm at seven days, 10—35 mm at 14 days (Fig. 2, G), 56—90+ mm at 10 weeks, fragile at first, then tough, adherent, slightly raised at the center, elsewhere compactly downy to felty and floccose, becoming thinner toward the margin, with the margin raised, often lobed, and the edge even, the mat often with deep radial wrinkles or folds that cause it to have a sectored appearance; mat entirely white to "light buff" or "avellaneous"; the agar under the inoculum block stained "Sayal brown"; culture having a distinct, sharp, fruity odor; the oxidase reaction negative on gallic acid and tannic acid media (Fig. 2, H, I), or sometimes weakly positive on gallic acid medium, the mycelium showing a trace of growth on gallic acid medium within seven days and forming a mat 18—60 mm in diameter at 10 weeks, no growth on tannic acid medium.

In test-tube cultures at 28 days the mat covering the diamalt agar slant and occasionally extending as much as 20 mm down the cylinder, forming a downy-felty floccose growth most profusely developed around the inoculum block, becoming thin, appressed, and translucent near the margin; the mat "light buff" or "olive buff"; the reverse side of mat "snuff brown" in the region of the inoculum.

Microscopic Characteristics: All hyphae (Fig. 3, C) staining with phloxine, (1—) 2—2.5 (—3) μ in diameter, sparsely branched, with numerous clamp connections formed as loops; hyphal coils usually not conspicuous at 14 days but common at six weeks; old cultures including hyphae with deeply staining portions having slightly thickened colorless walls; colored portions of the mat including staining hyphae with slightly thickened pale-brown walls.

Temperature Relations: The optimum growth temperature was 22° C, the minimum less than 8°, and the maximum approximately 30° (Table I). Cultures were killed by incubation at 34° for 21 days.

Cultures Studied: Cultures from Colorado studied as the basis for describing cultural characteristics include: F. P. 104558-Sp, F. P. 104726-Sp, F. P. 105070-Sp, F. P. 105071-Sp, and F. P. 105208-Sp, all on *Picea engelmannii*; Colo. 112, F. P. 94290-R, F. P. 104200-R, and F. P. 105116-Sp, all on *Pinus contorta*.

Remarks: Cultural aspects useful in recognizing *P. luna* include slow or very slow growth, tough mat, usually entirely negative oxidase reaction with a trace of growth on gallic acid medium but none on tannic acid medium, formation of clamp connections, and the appearance of hyphal coils that become more conspicuous with increasing age of the culture.

PENIOPHORA POLYGONIA

(Figs. 1, D; 2bis, J-L; 3, D; 7, A-D)

Peniophora polygonia (PERS. ex. FR.) BOURD. & GALZ., Hym. de France 320. 1928.

Thelephora polygonia PERS. ex FR., Syst. Myc. 1: 444. 1821.

Thelephora maculaeformis FR., Syst. Myc. 1: 454. 1821.

Corticium polygonium (PERS. ex FR.) FR., Epicr. 564. 1838; PERS., Roemer Neues Mag. Bot. 1: 110. 1794.

Cryptochaete polygonia (PERS. ex FR.) KARST., Bidr. K. Finlands Nat. och Folk 48: 408. 1889.

Aleurodiscus polygonius (PERS. ex FR.) HÖHN. & LITSCH., Öst. bot. Z. 58: 475. 1908.

Gloeocystidium polygonium (PERS. ex Fr.) HÖHN. & LITSCH., Wiesner Festschr. 69. 1908.

Gloeocystidium polygonium HÖHN. & LITSCH. var. *fulvescens* BRES., Mycologia 17: 69. 1925.

Basidiocarp Description

Macroscopic Characteristics: Basidiocarp (Fig. 1, D) originating as a tubercle that erupts from the woody substratum, then spreading and becoming confluent with adjoining basidiocarps to form a relatively thin, adnate, orbicular sheet with the margin either appressed to the substratum or free; hymenial surface "pale vinaceous-fawn" to "pale vinaceous-drab" or "light brownish vinaceous", or ranging to "fawn color" and "cacao brown" or "vinaceous-russet", often with a superficial grayish pruinose bloom.

Microscopic Characteristics: Hyphae (Fig. 7, A) characteristically having thick gelatinous-appearing walls not staining in phloxine solution, with clamp connections revealed especially by the characteristic configuration of the staining cell contents at the septa; dendrophyses (Fig. 7, B) recognizable principally in the very young hymenium; gloeocystidia (Fig. 7, C) characteristically pyriform, but somewhat variable, with walls hyaline and slightly thickened, contents granular to guttulate, approximately 50—60 or more × 15—25 μ; basidia 4-sterigmate, apparently collapsing soon after spore formation; spores (Fig. 7, D) curved-cylindrical, smooth, hyaline, non-amyloid, 10—11 × 3 μ.

Culture Description

Key Pattern: B-P-I-1-10-14; A-P-I-1-10-14-16.

Growth Characteristics: Growth on 2.5 % diamalt agar at 25° C moderately rapid, with mat 90+ mm in diameter at 14 days (Fig, 2 bis, J), firm to tough, adherent, forming a raised floccose-woolly growth over and around the inoculum block, then spreading outward as a flat cottony growth marked by radiating strands and appearing as a fine, translucent, nodulose intertwining of hyphae on the surface of the medium, becoming loose-woolly at the margin and frequently rolled up along the side of the dish, with small, smooth, waxy, "cinnamon brown" hymenial areas occasionally developing in the inoculum region at four to six weeks; mat at first entirely white, within 14 days becoming pale "cinnamon buff" at the center, elsewhere "cartridge buff"; the agar beneath the mat "vinaceous-russet" to "cameo brown"; culture having a mild fruity odor; oxidase reaction positive, with mycelial growth profuse on both gallic acid and tannic acid media (Fig. 2 bis, K, L).

In test-tube cultures at 28 days the mat covering the diamalt agar slant and extending to base of cylinder, with flat cottony strands radiating from the inoculum block and forming a floccose, reticulate growth over the agar surface, becoming raised in a loose-woolly fringe at the boundary between agar and test tube; the mat in general "cartridge buff", but with the thicker areas "tawny olive", the agar under the mat stained "pecan brown" to "Vandyke brown"; culture odorless.

Microscopic Characteristics: All hyphae (Fig. 3, D) staining with phloxine, (1.5—)2—3.5 (—6) μ in diameter, with numerous large simple clamp connections, the superficial hyphae long and infrequently branched, the submerged hyphae with numerous short, blunt branches and with various sizes of torulose swellings; many hyphae in the center of the mat heavily encrusted by large crystals at 14 days; gloeocystidia developing in some cultures within four to six weeks, pyriform, 20—25 × 14—18 μ, with the contents staining deeply and including numerous oil globules.

Temperature Relations: The optimum growth temperature was approximately 26° C, the minimum less than 8°, and the maximum approximately 32° (Table I). Cultures were killed by incubation at 36° for 10 days.

Cultures Studied: Cultures studied as the basis for describing cultural characteristics include: From Colorado — F. P. 104959-Sp, F. P. 105068-Sp, F. P. 105095-Sp, F. P. 105097-Sp, Colo. 54-311-R, and Colo. 56-283-R; from Alaska (Ester Dome) — F. P. 109123-Sp, All cultures were originally isolated from basidiocarps or rot from *Populus tremuloides*.

Discussion

In placing *P. polygonia* in *Aleurodiscus*, HÖHNEL & LITSCHAUER (15) and PILÁT (23) emphasized that it has dendrophyses. In herbarium material utilized during the present study, dendrophyses were difficult to demonstrate except in very young specimens and did not seem to be very distinctive. ERIKSSON (13) included this species in *Peniophora* sect. *Coloratae*. Although it does not have encrusted cystidia, it seems to show a close relationship to the *Coloratae* and is probably best situated in that group.

PENIOPHORA RUFA

(Figs. 1, E; 2 bis, M-O; 3, E; 7, E-I)

Peniophora rufa (FR.) BOIDIN, Bull. Soc. Myc. France 74(4): 443. (1958) 1959.

Thelephora rufa FR., Elench. 1: 187. 1828.

Tubercularia pezizoides SCHW., Amer. Phil. Soc. Trans. (n. ser.) 4: 301. 1832.

Stereum rufum (FR.) FR., Epicr. 553. 1838.

Hypocrea richardsoni BERK. & MONT., Grevillea 4: 14. 1875.

Xerocarpus rufus (FR.) KARST., Bidr. K. Finlands Nat. och Folk 37: 135. 1882.

Cryptochaete rufa (FR.) KARST., Bidr. K. Finlands Nat. och Folk 48: 408. 1889.

Corticium pezizoideum (SCHW.) SCHRENK, Torrey Bot. Club Bull. 21: 388. 1894.

Sterellum rufum (FR.) JOHN ERIKSS., Symb. Bot. Upsal. 16(1): 120. 1958.

Basidiocarp Description

Macroscopic Characteristics: Basidiocarp (Fig. 1, E) tuberculiform, waxy-cartilaginous; hymenial surface "Corinthian red" to "vinaceous-brown", often with a grayish pruinose bloom.

Microscopic Characteristics: The microscopic structure as described by LENTZ (21), with hyphae (Fig. 7, E) having thick gelatinous-refractile walls, with clamp connections; gloeocystidia (Fig. 7, I) 50—190 × 7.5—27.5 μ; cystidia (Fig. 7, G) inconspicuous except for the encrusted apices having the appearance of small crystalline masses in the hymenium, approximately 35 × 4.5—7.5 μ; basidia (Fig. 7, F) 4-sterigmate, 30—47.5 × 3.5—5.5 μ; spores (Fig. 7, H) curved cylindrical or suballantoid, smooth, hyaline, nonamyloid, 6—8.5 × (1.5—) 2 (—3) μ.

Culture Description

Key Pattern: A-P-I-1-10-16; B-P-I-1-5-6-10-14-16.

Growth Characteristics: Growth on 2.5 % diamalt agar at 25° C moderately rapid, with mat having a diameter of 38—64 mm at seven days, 90+ mm at 14 days (Fig. 2 bis, M), tough, adherent, usually with the surface growth loose, floccose-woolly, indistinctly reticulate, extending entirely across and up the side of the dish and recurving as a loose, fragile fringe of hyphae, or sometimes with the growth compact-woolly over and around the inoculum block, spreading as a thin, collapsed, downy, reticulate zone, bordered by a marginal zone characterized by scattered, loose, woolly spheres of mycelium 2—4 mm in diameter, with fertile hymenial discs developing along the margin of some cultures within 14 days and forming extensive marginal hymenial areas within three to four weeks; upper suface of mat white, or with a tinge of "light buff" at the center; the underside of mat "ochraceous-tawny" at the center, with the surrounding zone "light ochraceous-salmon", culture odorless, or with a faint sweet odor; oxidase reaction strongly positive on gallic acid and tannic acid media (Fig. 2 bis, N, O), with a trace of growth on gallic acid medium or with thin, flat, "cinnamon brown" fans of mycelial growth spreading out from one side of the inoculum block, with the growth on tannic acid medium varying from a trace to a thick floccose-woolly mat 14—45 mm in diameter and resembling the growth on diamalt agar.

In test-tube cultures at 28 days the mat covering diamalt agar slant and extending down cylinder to bottom of tube, forming a densely woolly to felty growth over and around the inoculum block and spreading as a flat-downy to appressed translucent growth with scattered raised spheres of mycelium 2—4 mm in diameter, on the cylinder forming a flat-cottony, reticulate, nodulose growth; the mat "pale olive buff" or "light buff"; the reverse side of mat "ochraceous-tawny" to "cinnamon brown"; culture odorless.

Microscopic Characteristics: Many hyphae staining with phloxine, septate, with large simple clamp connections, (0.5—) 2—3.5 (—7.5) μ in diameter, with numerous short, blunt branches; other hyphae non-staining at the end of 14 days, or with narrow staining lumina surrounded by thick, hyaline, non-staining walls (Fig. 3, E), with occasional clamp connections, (2—) 3.5—4 (—8) μ in diameter, rather infrequently branching; the central portion of the mat including many heavily encrusted hyphae within 14—21 days; gloeocystidia appearing in cultures at 14 days and abundant within 21—28 days, deeply staining, with thick hyaline walls, 30—70 × 4.5—6.5 μ; cystidia appearing in the hymenium but not abundant, hyaline, with encrusting crystalline matter limited to their apical regions, 20—35 × 3.5—4 μ, very slightly exserted; basidia formed in many cultures at 14 days, 4-sterigmate, clavate, 30—60 × 3.5—5 μ; basidiospores cylindrical or curved-cylindrical, 6—7.5 × 2—2.5 μ.

Temperature Relations: The optimum growth temperature was 24° C, the minimum 8—10°, and the maximum 34° (Table I). Cultures were killed by incubation at 36° for 10 days.

Cultures Studied: Cultures from Colorado studied as the basis for describing cultural characteristics include: F. P. 104878-Sp, F. P. 105072-Sp, F. P. 105096-Sp, and F. P. 105098-Sp, all from *Populus tremuloides* where *P. rufa* occurs apparently as a saprobe.

Remarks: Cultures of *P. rufa* may be distinguished from those of *P. polygonia* by several contrasting characteristics. *P. rufa* forms a loose, floccose-woolly mat that is white or has a tinge of "light buff", whereas *P. polygonia* forms a flat, spreading, reticulate mat that is usually "cartridge buff" at 14 days. *P. rufa* develops a "light ochraceous-salmon" stain on the underside of the mat on diamalt agar and forms only a trace of growth on gallic acid medium, while *P. polygonia* produces a distinctive "vinaceous-russet" stain on the underside of the mat and usually grows well on both gallic acid and tannic acid media. In cultures, at the end of two weeks, *P. rufa* has numerous branching hyphae with clamp connections, thick walls, and narrow staining or occasionally empty lumina. These thick-walled hyphae are not found in cultures of *P. polygonia* at the end of two weeks although they are abundant in older cultures.

Discussion

P. rufa seems to be closely related to *P. polygonia*. Evidences of this relationship have previously been cited by Lentz (21). Further evidence, such as non-amyloid spores in both species, merely tends to substantiate the impression that they are related. Both species may be regarded as belonging in *Peniophora* sect. *Coloratae*. Thus BOIDIN'S (4) new combination, *P. rufa*, is accepted for the fungus previously called *Cryptochaete rufa* (FR.) KARST. by LENTZ (21) or *Sterellum rufum* (FR.) JOHN ERIKSS. by ERIKSSSON (14).

SUMMARY

Descriptions of basidiocarps and cultures are provided for *Helicobasidium corticioides*, *Corticium radiosum*, *Peniophora luna*, *P. polygonia*, and *P. rufa*, which are associated with decay of forest trees in Colorado. The first three species attack conifers; *P. polygonia* and *P. rufa* occur on aspen. Noteworthy taxonomic characteristics are discussed and are illustrated by photographs and drawings.

LITERATURE CITED

1. BANDONI, R. J., 1955. A new species of *Helicobasidium*. Mycologia **47:** 918—919.
2. BIGGS, R., 1937. The species concept in *Corticium coronilla*. Mycologia **29:** 686—706.
3. BOIDIN, J., 1951. Sur la présence des anses d'anastomose dans le carpophore de quelques Basidiomycètes résupinés. C. R. Acad. Sci., Paris **233:** 824—826.
4. —, [1958] 1959. Hétérobasidiomycètes saprophytes et Homobasidiomycètes résupinés. IV. — Les *Peniophora* section *Coloratae* B. & G. a dendrophyses. Bull. Soc. Myc. France **74** (4): 436—481.
5. BOURDOT, H. & A. GALZIN, [1927] 1928. Hyménomycètes de France. 761 pp. Sceaux.
6. BURT, E. A, [1925] 1926. The Thelephoraceae of North America. XIV. *Peniophora*. Ann. Missouri Bot. Gard. **12:** 213—357.
7. —, 1926. The Thelephoraceae of North America. XV. *Corticium*. Ann. Missouri Bot. Gard. **13:** 173—304.
8. CUNNINGHAM, G. H., 1954. Thelephoraceae of New Zealand. Part III: The genus *Corticium*. Trans. Roy. Soc. New Zealand **82**(2): 271—327.
9. DAVIDSON, R. W., W. A. CAMPBELL & D. J. BLAISDELL, 1938. Differentiation of wood-decaying fungi by their reactions on gallic or tannic acid medium. J. agric. Res. **57**(9): 683—695.
10. —, — & D. B. VAUGHN, 1942. Fungi causing decay of living oaks in the eastern United States and their cultural identification. U. S. D. A. Tech. Bul. 785.
11. — & T. E. HINDS, 1958. Unusual fungi associated with decay in some forest trees in Colorado. Phytopathology **48**(4): 216—218.
12. DONK, M. A., 1956. Notes on resupinate Hymenomycetes — III. Fungus **26:** 3—24.
13. ERIKSSON, J., 1950. *Peniophora* Cke. sect. *Coloratae* Bourd. & Galz. A taxonomical study with special reference to the Swedish species. Symb. Bot. Upsalienses **10**(5): 1—76.
14. —, 1958. Studies in the Heterobasidiomycetes and Homobasidiomycetes — Aphyllophorales of Muddus National Park in North Sweden. Symb. Bot. Upsalienses **16**(1): 1—172.
15. HÖHNEL, F. VON & V. LITSCHAUER, 1908. Norddeutsche Corticieen. Öst. bot. Z. **58:** 441—444, 471—478.
16. JACKSON, H. S., 1948. Studies of Canadian Thelephoraceae II. Some new species of *Corticium*. Canad. J. Res., C, **26:** 143—157.

17. Käärik, A. & E. Rennerfelt, 1957. Investigations on the fungal flora of spruce and pine stumps. Meddel. Stat. Skogsforskningsinstitut [Sweden] **47**(7): 1—88.
18. Karsten, P. A., 1876. Mycologia Fennica. Pars Tertia. Basidiomycetes. Bidr. K. Finlands Nat. och Folk **25:** I—X, 1—377.
19. —, 1881. Enumeratio Thelephorearum Fr. et Clavariearum Fr. Fennicarum, Systemate Novo Dispositarum. Rev. Myc. **3**(9): 21—23.
20. —, 1894. Kritisk öfversigt af Finlands basidsvampar. II. Bidr. K. Finlands Nat. och Folk **54:** 157—186.
21. Lentz, P. L., 1955. *Stereum* and allied genera of fungi in the Upper Mississippi Valley. U. S. D. A. Agr. Monograph 24.
22. Lyman, G. R., 1907. Culture studies on polymorphism of Hymenomycetes. Proc. Boston Soc. Nat. Hist. **33**(4): 125—209.
23. Pilát, A., 1926. Monographie der mitteleuropäischen Aleurodiscineen. Ann. Myc. **24:** 203—230 .
24. Ridgway, R., 1912. Color Standards and Color Nomenclature. 43 pp. Washington.
25. Rogers, D. P., 1944. The genera *Trechispora* and *Galzinia* (Thelephoraceae). Mycologia **36:** 70—103.
26. — & H. S. Jackson, 1943. Notes on the synonymy of some North American Thelephoraceae and other resupinates. Farlowia **1**(2): 263—328.

CRITICAL SURVEY OF THE PRESENT STAND OF THE PRODUCTION OF PERFECT STAGE OF ORGANS OF FRUCTIFICATION IN DERMATOPHYTES.

by

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(28.III.1960)

Attempts to produce or to observe the perfect stage of the organs of fructification in dermatophytes began six decades ago. The first observations were reported by Matruchot & Dassonville in 1899. Few papers alleging the successful production of the perfect stage of fructification in any form-genus of dermatophytes have been published since then. No two authors have used the same techniques. Moreover, none of the allegedly successful attempts ever were repeated either by their authors or by subsequent investigators. Some authors have described their techniques inadequately. Others presented their techniques, but the description and particularly the illustrations of the "perfect stage," including the evolutive steps which led from the spore to the perithecium, were unsatisfactory.

Furthermore, there exists a basic confusion in these publications which require clarification from the beginning. In discussing the "perfect stage of fructification" in dermatophytes, two facts have to be clearly kept apart. Some reports deal with instances in which this "perfect stage of fructification" of a dermatophyte already appeared in the primary cultures, starting from a fungus infected hair, in tinea capitis, for instance. In these instances, either the conidial and perithecial stages were present simultaneously or the conidial stage appeared only in vestigeal form and the perfect (perithecial) stage prevailed. These observations are extremely valuable because they show that the dermatophytes at one time had a perfect stage which disappeared during adaptation from a saprophytic existence to parasitic life in animal tissues. As a result of this "adaptation for survival" the perithecial stage for the most part vanished and a conidial stage took its place in parasitic life of the same fungus. The few publications on this matter allow for the conjecture that dermatophytes in their perfect stage occur more often than has generally been appreciated.

Other investigations began with the conidial stage and aimed at the production of the perithecial stage in dermatophytes. These have been unsuccessful in producing the perfect stage on artificial culture mediums. The core of the problem lies in these investigations.

The basic problem of future investigations is to find reliable methods by which dermatophytes of which only the conidial stage is presently known, can be forced to produce the perithecial stage. Whether this is possible in all four form-genera, i.e., *Trichophyton*, *Microsporon*, *Achorion* and *Epidermophyton*, only the future can reveal. It would also be theoretically possible that in any of the form-genera only a few strains found incidentally, can be forced to produce the perithecial stage because cytologic and/or genetic changes in the conidial stage of these dermatophytes have progressed so far that a return to the perfect stage has become impossible. Cytologic and/or genetic studies may shed light on this problem.

Matruchot & Dassonville, 1899

The investigations of these authors, though highly interesting, are most peculiar. The whole study is based upon a single strain of *Trichophyton* sp., isolated in an epidemic of herpes tonsurans in horses. The species remained undetermined. However, they did not leave any doubt regarding the pathogenicity of this strain; its pure culture was inoculated in man, guinea pig, rabbit and re-inoculated in the horse; they always obtained typical lesions. They have never had, nor apparently never intended to obtain a perithecial stage of this single strain of *Trichophyton* sp. Their conclusion, that the form-genus of *Trichophyton* and probably all dermatophytes (*Microsporon, Achorion, Epidermophyton*) belong to the Gymnoascaceae, particularly to Ctenomyces, is based solely upon the similarity of external characteristics between the imperfect organs of fructification of this single strain of *Trichophyton* sp. and those of the Gymnoascaceae, especially *Ctenomyces*, (aleuria, chlamydospore, arthrospore, fuseau, spiral, etc.). It is most amazing that in the following decades taxonomic systems of dermatophytes have evolved and have stamped species "*Gymnoascus*" . . ., "*Ctenomyces*"..., without anyone ever having seen, let alone having produced the perithecial stage of these fungi.

In a later study, Matruchot & Dassonville reiterated their opinion that the imperfect stage of fructification is sufficient to attach the dermatophytes, in particular, the form-genus *Trichophyton*, to the family of Gymnoascaceae and here, foremost to the conidial form of *Ctenomyces*. It is the *Ctenomyces serratus* Eidam which particularly reveals homologous features with those found in *Trichophyton* sp.: lateral and intercalary chlamydospores, fuseau, pectinate organs, spirals, etc. They admitted, however, that in a natural classification of fungi the decisive criterion is the perfect organ of fructification. The form and structure of the asci and ascospores give the taxonomic characteristics of the first order, but the perfect stage of fructification for the dermatophytes, they added, is yet to be found.

Even more curious is the final contribution of these authors with regard to the question concerning the perfect stage of fructification in dermatophytes. This paper deals with *Eidamella spinosa*, a dermatophyte which produces perithecia. This fungus was discovered in the culture of hairs of a dog which had contracted herpes tonsurans. They considered this fungus the causative agent of the skin lesions because among others, they found it repeatedly in the culture. This fungus produced perithecia in the primary culture and was regarded by these authors to be the first dermatophyte discovered which produced perithecia. They believed that their hypothesis, that all the dermatophytes belong to the family of Gymnoascaceae, was confirmed by this finding. *Eidamella spinosa*, MATRUCHOT & DASSONVILLE [1]) could be cultured on any routine laboratory medium as well as on carrots, parsnips (*Pastinaca sativa*), on horse and dog hairs, etc. The culture was first white, but when the perithecia were formed, it turned greyish, then greyish-black. In the substratum it produced a purple pigment resembling that of "some other dermatophyte." Perithecia formed in great number on aerial mycelia. The peridium was not submembranous as in *Ctenomyces*; it was even more loose than in *Gymnoascus*. In the center of this loose perithecium the asci were produced in "grappe" at the internal face of the perithecium. The asci were pedicellate. They all matured almost at the same time and were visible through the wall of the perithecium between the ascogenous hyphae. Asci were eight-spored; they, as well as the ascospores, were colorless. The membrane of the ascus was very ephemeral. One often observed eight spores stuck together, due to a mucilaginous material without a visible ascus wall. Attempt at inocculation with a pure culture of *Eidamella spinosa* to cause similar lesions as in the dog, failed. They ascribed this failure to the fact that the perithecial form is far less virulent than the conidial stage in Gymnoascaceae.

It is remarkable that research workers in this field accepted the conclusions of MATRUCHOT & DASSONVILLE, that the dermatophytes. in a natural system, belong to the Gymnoascaceae. Through six decades no one ever challenged this tenet. First of all, the theory of MATRUCHOT & DASSONVILLE was based upon an hypothesis, derived from homologies and analogies, but not upon facts, that is, not on the actual observation of the production of the perfect stage of fructification, the perithecia. Secondly, they had no evidence as to whether *Eidamella spinosa*, found in a lesion on a dog together with other fungi, was the real cause of the tinea. Despite this, they used *Eidamella spinosa* as the final proof of their hypothesis, that all dermatophytes belong to the Family Gymnoascaceae, Genus *Ctenomyces*.

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[1]) This fungus later was lost and up to date has not been recovered.

We may add that MATRUCHOT & DASSONVILLE did not in a single instance obtain the perfect organs of fructification, the perithecia, in any of the four form-genera: *Trichophyton*, *Microsporon*, *Achorion*, and *Epidermophyton*.

CHALMERS & MARSHALL, 1914

In 1914, CHALMERS & MARSHALL gave an account of a *Trichophyton endothrix* which they found to be the cause of common ringworm on the heads of school children in Khartoum and Obdurman in the then Anglo-Egyptian Sudan. They called this fungus, *Trichophyton currii*, CHALMERS & MARSHALL, 1914.

The growth of *Tr. currii* was white on Sabouraud's maltose agar, but if the culture was kept for several months it gradually became black. If the growth, produced at atmospheric temperature in one of Kitasato's flasks, i.e., in a thin layer of the medium, was later examined, a number of small black dots could be seen. In the average they measured 0.5 mm and were composed of interlocked hyphae, with thickened, greenish-black walls. Their contents showed hyphae and cells and the spaces between them were filled, in the fresh state, with a "fatty material." The "black dots" first appeared when the cultures were about two months old.

In their younger condition when they, with some of the surrounding hyphae, were planted on Sabouraud's or other mediums, typical trichophytic growths were produced, apparently indistinguishable from those originally obtained from hair. They gave the same lesions when inoculated into animals as those produced by inoculations direct from patients' heads or from fresh cultures.

Due to their structure and appearance they considered the "black dots" as perithecia. Since the walls of these "perithecia" were simply composed of interlocked hyphae, they considered the fungus giving rise to them as belonging to the Hemiascomycetes and to the Family Gymnoascaceae BARANETZKY 1872.

There is no doubt that CHALMERS & MARSHALL in *Tr. currii* had a fungus which produced a perfect, even though primitive form of organ of fructification in the primary culture and on routine Sabouraud's maltose agar. They did not require an artifice (special mediums, animal substances such as feather, leather, horn, etc.) to force the fungus in its saprophytic stage to produce perfect organs of fructification. By their experiment of re-inoculation with fungus growth with perfect fructification they apparently proved that *Tr. currii* was the true cause of tinea capitis. This fungus produced and continued producing, in addition to the imperfect form of fructification, also perfect organs of fructification on routine mediums.

The finding of CHALMERS & MARSHALL, however, did not prove that any other member of the form-genus *Trichophyton* of SABOURAUD had a similarly perfect stage of fructification, or that these members represented the conidial forms of any higher fungi which would necessarily also belong to the Gymnoascaceae. They left the question whether any member of the form-genus *Trichophyton* of SABOURAUD does or does not form perfect organs of fructification, entirely open. On routine mediums, they certainly do not form the perfect stage of fructification spontaneously in the primary culture. Whether or not ways can be found to uniformly force all the members of form-genus *Trichophyton* of SABOURAUD to produce a perfect stage of fructification remains an unsolved question.

However, CHALMERS & MARSHALL's description leaves ample room for doubt as to what they really observed. We quote these authors verbatim: "The structure and appearance of the black dots confirms us in the view that they are Perithecia. Further, the fact that they are simply composed of interlocked hyphae indicated that the fungus giving rise to them belongs to the Hemiascomycetes and to the Family Gymnoascaceae BARANETZKY 1872. Their origin appears to us to be simply thickened hyphae wrapped around other hyphae which break up into fatty bodies (sic!) and the ovoidal bodies mentioned above. These ovoidal bodies are, in our opinion, asci which have failed to produce ascospores, nor is the reason of this difficult to understand, as it is simply a stage of the adaptation from the saprophytic, or the parasitic, existence on a plant to the more perfect parasitic condition on a man."

"We are further of the opinion that *Eidamella spinosa*, MATRUCHOT & DASSONVILLE, 1901, indicated the least specialized from of *Trichophyton* known, in that it develops ascospores in the asci. *Trichophyton currii* appears to us to have advanced (sic) a step further along the line of parasitic adaptation in that it has ceased to develop the ascospores (sic), and, moreover, has begun to develop fusiform bodies and spiral loops . . . In our opinion, a step further in parasitic existence is indicated by the appearance in the cultures of *T. currii* of a very few spindle-shaped bodies and a few spirals . . ."

Even with lenient judgment one may have doubt about what CHALMERS & MARSHALL had seen. Ascomycetes with asci, but without any ascospores? How did this fungus propagate itself? Simply by means of mycelial elements or by the conidial stage? The critical analysis of the papers of CHALMERS & MARSHALL does not lead to the unqualified conclusion that they really had the perfect form of fructification of a *Trichophyton* before them, a *Trichophyton* (*Tr. currii*) which "was closely related to a possible parental form of the Crateriform and the Acuminate groups of the Endothrix division of the Genus *Trichophyton*."

A. Wilenczyk, 1926

This author, in three papers, reported upon successfully causing members of the form-genus *Trichophyton* to form perithecia. He experimented with three strains of *Tr. granulosum* and one of *Tr. violaceum*. He gave in detail his technique of the "hanging drop culture." According to his experience, the flow of oxygen, the proper temperature, (first at 37° C degrees in an incubator and after 5—6 passages, at room temperature) and finally, the complete drying of the culture medium (Sabouraud's milieu d'épreuve), were apparently the main conditions for the production of the perfect stage of fructification.

Wilenczyk probably never observed perithecia formation in any Ascomycetes (such as *Chaetomium*, *Allescheria boydii*, etc.). Otherwise, he would not have given such a "stuttering" description of his observation. His description is couched in naive, wholly unmycological terms. One sentence is enough to show the most dilettante approach to the problem. "J'ai constaté en même temps que, dans certaines conditions, le *trichophyton* commence à former des asques qui apparaissent à la place (sic!) des perithèces, des conidies, et des chlamydospores." ("I observed at the same time, under certain conditions, that the *trichophyton* begins to form asci which appear at the place of perithecia, of conidia and of chlamydospores!!")

Thus, the ascus formed before a perithecium developed! He then observed spore formation in the asci in the number 2, 4, 6, 8! There is nothing more constant in a given species of Ascomycetes than the number of ascospores in any one ascus. Their number is always constant. It is unnecessary to further analyze these papers. The illustrations show that the author never saw how a perithecium is formed. If one were to try to guess what species of fungus he had at hand, what kind of conidial formation he mistook as asci, the nearest guess would be that he had *Alternaria* in his preparation. (Cf. figs. 1 and 4 in paper 1927).

So we may conclude that Wilenczyk's endeavor to cause the perfect stage of fructification in the form-genus *Trichophyton* was unsuccessful.

A. Nannizzi, 1926

Nannizzi became the first to prove the hypothesis of Matruchot & Dassonville, that there is a morphological and biological relationship between the Dermatophytes and the Gymnoascaceae. The starting point of his investigations was the simple observation that if dermatophytes are cultured on "natural mediums" with physico-chemical characteristics of human and animal tissue, they will produce strong mycelia which, at a certain point of their development, will break up into arthrospores ("mycelial spores", NANNIZZI). This is the same picture that can be observed in a trichophytic lesion or in the trichophytic hair. If, however, we culture the same fungi on "artificial mediums" containing more or less carbohydrate, certain new structural elements will appear, resembling spiral, pectinate hyphae, candelabrum, fusiform chlamydospores, grapes, nodular organs — sometimes even rudimentary features of pycnidia. He considered these structures as "teratologic habitus" which have no parallel among other fungi living in a natural state: saprophytic or parasitic. He considered "artificial mediums" the greatest obstacle in the quest for the existence of higher organs of fructification in Dermatophytes.

Therefore, he tried to culture certain Dermatophytes on "natural mediums" such as feathers of fowl, bones of mammals, hairs of guinea pigs, as well as human hair, on pieces of leather. He maintained these cultures in the dark, in humid atmosphere at ambient temperatures. His technique was simple. He put water into the bottom of Roux test tubes. This maintained a constant humidity in the "natural mediums" which he placed above the water. After sterilization of the prepared tubes in an autoclave he inoculated the mediums with various *Trichophytons*. The test tubes were kept for a few days in an incubator at 25° C in order to facilitate development of the fungi. They then were left at the temperature of the laboratorium.

The following "species" were examined by this method: *Tr. radiolatum* SAB., *Tr. asteroides* SAB., *Tr. denticulatum* SAB., *Tr. felineum* R. BLANCH., *Tr. equinum* MATRUCHOT, *Tr. granulosum* SAB., *Microsporon lanosum* SAB.

Five months after inoculation on hen feathers or on hairs, whitish globules, one third to one half mm in diameter, appeared on the surface of the vegetative mycelium. These globules consisted of a central core composed of "grappes," densely and repeatedly subdivided. They contained a great number of elements such as globose, terminal and lateral aleuria, each being provided with a large refrigent nucleus. These globules were surrounded by a web of loose and fragile hyphae, branching and anastomosing, from which a variable number of hyphae radiated, ending in curls or spirals.

The "cover" of these globules revealed a "perfect similarity" to the peridium of the Gymnoascaceae, (especially in the gen. *Myxotrichum*) while the dense "grappes" and spherical aleuria "reminded one of ascogenous hyphae and asci." Since the structures within the "globules" are aleuria and not true asci, these globular organs need to be considered as "imperfect peridia" (perithecia) which should appropriately be called "pycnidia."

In *Tr. asteroides* SAB. pycnidia developed late and only a few on leather. *Tr. denticulatum* SAB. developed abundant pycnidia on feathers and hairs. Here the pycnidia first appeared when the stroma of the culture became exhausted. The cover of the pycnid was lanuginose, loosely interwoven. The external hyphae were frequently arcuate, unilaterally branched and/or retroflexed. The internal hyphae were branched, carrying very dense, ovoid and pyriform aleuria (pycnospores). These pycnidia resembled to a great extent the peridium of the Gymnoascaceae, particularly those of the gen. *Gymnoascus*.

In *Tr. felineum* R. BLANCH. pycnidia did not appear on leather or on feathers, but were rarely observed on hairs about four months following inoculation. They were irregular in shape, variable in size, with aleuria (pycnospores) which were rounded and provided with a cover (peridium) which was extremely loose and evanescent, without appendix.

Tr. equinum MATRUCHOT grew well on leather, but not on feathers or hairs. Pycnidia appeared on adult mycelium as white globules provided with few and tortuous appendices, containing dense "grappes" of aleuria (pycnidia).

In *Tr. granulosum* SAB. and in *M. lanosum* SAB. pycnidia have never been observed.

NANNIZZI also stated that prior to his investigations only one dermatophyte(?), *Eidamella spinosa* MATRUCHOT & DASSONVILLE, had been found which produced perfect perithecia with asci.

The reasons for doubting that this *Eidamella spinosa* was the real cause of the fungus lesion, have already been stated.

Macroconidia or pluriseptate fuseau can be found in abundance in some *Microsporon*, in *Achorion*, in *Trichophyton* (the *Gypseum* group) and in *Epidermophyton*. Uni- and pluricellular macroconidia occur in Gymnoascaceae in *Ctenomyces serratus* and *Myxotrichum uncinatum*.

NANNIZZI came to the following conclusions: the dermatophytes during their parasitic life appear in a particular, highly reduced form of adaptation, i.e., they consist of almost linear and little branched, mycelial filaments which break up in arthrospores and chlamydospores. On culture mediums they develop a richer and more branched mycelium on which appear aleuria, simple or in "grappes," macroconidia (fuseau), spiral and pectinate hyphae, racquet, terminal claves, candelabrum and nodular bodies. These imperfect organs of fructification may appear singly in certain species, or all together in the same species. If these fungi are cultured on substratum of animal origin such as feathers, hairs, leather or bone, they may produce in their saprophytic life organs of fructification of higher order, analogous to the perithecia of Ascomycetes belonging to the family Gymnoascaceae. The difference is that the dermatophytes do not produce asci but simply pycnospores which have the equivalent value of pycnidia. Thus, on substratum of animal origin pycnidia were produced in *Tr. radiolatum* SAB., *Tr. asteroides* SAB., *Tr. denticulatum* SAB. and *Tr. felineum* R. BLANC.

The analogy of the pycnidia with the perithecia of the Gymnoascaceae is conclusively demonstrated by the culture of some of these Ascomycetes on artificial culture mediums, i.e. *Ctenomyces serratus* EID., *Arachniotus candidus* (EID.) SCHROET, *Gymnoascus Reesii* BARANETZKY, *Myxotrichum chartarum* KUNZE, *Myxotrichum uncinatum* (EID.) SCHROET. On artificial mediums these ascomycetes appear identical with certain dermatophytes on the same substratum. Like the dermatophytes, these ascomycetes produce on artificial mediums the same aleuria, simple or in "grappes," arthrospores, chlamydospores, macroconidia, racquet and spiral hyphae. The Gymnoascaceae in turn, lose their capacity to produce perithecia on artificial substratum. If they are again transferred to natural mediums of animal origin, they begin producing normal perithecia with asci and ascospores.

The analogy which exists between the pycnidia obtained in Dermatophytes and the perithecia of Gymnoascaceae led NANNIZZI to study the morphology and development of fungi belonging to this Family and to study in particular certain species of the genera *Ctenomyces*, *Myxotrichum*, *Arachniotus* and *Gymnoascus*, living either as saprophytes on natural substratum or cultured on artificial mediums.

The comparative study revealed that the different mycelial structures and the various elements of reproduction of the Dermatophytes can always be found in the Gymnoascaceae.

Racquet mycelia are most common in the form-genus *Microsporon*. However, it was found by OTA & LANGERON in *Tr. ochraceum* SAB. and in *Epidermophyton*. Among Gymnoascaceae they can be found in *Ctenomyces serratus* and *Myxotrichum uncinatum*

Pectinate hyphae were noted in *Microsporon* and *Achorion Schönleinii*, as well as — analogously — in *Ctenomyces serratus* and *Myxotrichum uncinatum*.

Spiral hyphae are found in *Trichophyton* sp., as well as in *Ctenomyces serratus* and *Myxotrichum uncinatum*.

Terminal claves occur in the greater part of Dermatophytes, in almost all the Gymnoascaceae, particularly in *Myxotrichum uncinatum* and *Ctenomyces serratus*.

Candelabrum (chandelier favique) occur in *A. Schönleinii*, in *Tr. album* and in *M. equinum*, as well as in submerged mycelia of *Myxotrichum uncinatum*.

Nodular organ is found in genera *Trichophyton*, *Microsporon* and *Achorion*, as well as in *Myxotrichum uncinatum*.

Aleuria (lateral sessile or pedunculate conidia), simple or in "grappes", occur in dermatophytes, as well as in Gymnoascaceae.

Arthrospores are present in all Dermatophytes, as well as in all Gymnoascaceae.

Chlamydospores are frequent in Dermatophytes as well as in Gymnoascaceae. Intercalary chlamydospores are frequent in

Arachniotus aureus and *Myxotrichum uncinatum*; terminal ones in *Myxotrichum chartarum*. Lateral chlamydospores are frequent in *A. Schönleinii* and *Quinckeanum* as well as in *Tr. album, violaceum, umbilicatum* and in *Myxotrichum uncinatum*.

The Dermatophytes must definitely be placed in the Family Gymnoascaceae BARANETZKY, concluded NANNIZZI, unifying in the sub-family Atelogymnoascaceae GRIGORAKI those genera whose perfect stage of fructification is not yet known, and in those of Gymnoascaceae CAST. & CHAL., those genera in which perithecia are already known.

Thus, NANNIZZI gave the following taxonomy of the dermatophytes:

Fam. Gymnoascaceae BARANETZKY 1872
Subfam. I Gymnoasceae CASTELLANI & CHALMERS, 1918
Sect. I Eugymnoasceae (NANNIZZI, 1926).
Myxotrichum KUNZE, 1823
Gymnoascus BARANETZKY, 1872
Ctenomyces EIDAM, 1880
Amauroascus SCHROETER, 1893
Arachniotus SCHROETER, 1893
Eidamella MATR. & DASSONV., 1901

Sect. II Nothogymnoasceae (NANNIZZI, 1926).
Myxotrichella SACC., 1892
Ateleothylax OTA & LANG., 1923

Subfam. II Atelogymnoasceae GRIGORAKI, 1924.
Trichophyton MALMST., 1848 (em. OTA & LANG., 1923).
Sabouraudites OTA & LANG., 1923.
Bodinia OTA & LANG., 1923
Endodermophyton CASTELLANI, 1909
Grubyella OTA & LANG., 1923
Epidermophyton LANG., 1879 (em. OTA & LANG., 1923).

It seemed to me necessary to present NANNIZZI's ideas, the trend of his investigations and his conclusions, since he was the first, and up to date the only investigator who had developed a technique of forcing certain dermatophytes to produce organs of fructification of higher order. Until his investigations have been repeated, we will have to accept his hypothesis that the Dermatophytes (in NANNIZZI's sense all dermatophytes) belong to the Gymnoascaceae. So far only CIFERRI & REDAELLI (cf. below) have attempted to duplicate NANNIZZI's investigations.

Furthermore, we must remember that NANNIZZI succeeded to substantiate his theory only in the case of a small group of dermatophytes. All fungi in which he was successful belonged to the form-genus *Trichophyton* and to the species which SABOURAUD had classified as *Trichophyton ectothrix microid*: *Tr. radiolatum, asteroides*, members of the *Gypseum* group; *Tr. denticulatum*, member of the *Niveum* group, and *Tr. felineum* which belongs to the *Trichophyton ectothrix megaspore*.

I believe that Nannizzi went to far in his generalization considering all dermatophytes to belong to the Gymnoascaceae. He never furnished any proof for this. One cannot know in advance, without definite evidence, whether or not members of the form-genera *Microsporon*, *Achorion* and *Epidermophyton* would, indeed, belong to the Gymnoascaceae.[1]) Nannizzi himself emphasized that in *Microsporon lanosum* Sab. he had never observed the production of pycnidia on natural substratum.

In 1927, Nannizzi reported on the successful production of perithecia in *Achorion gypseum* Bodin. The culture medium consisted of forest soil mixed with small pieces of old leather and hen feathers. On this medium the fungus showed, between February and October 1926, several periods of rest alternating with further growth. Only after these many months could characteristic nodular organs, and eventually typical perithecia be discovered on fertile mycelia. The peridium of these perithecia consisted of a web of abundantly branched hyphae. These were hyaline and smooth, surrounded by another layer of courser hyphae. They were arcuate; simple or branching, consisting of segments with thickened walls, echinulate, with granular contents. Inside these perithecia there were numerous asci which formed fairly dense "grappes", partly sessile, partly with short pedicles, globose or slightly oval. The asci contained eight ascospores, oval, hyaline, smooth, 4.5 by 3 μ in size. The membranes of the asci at maturity transformed into a mucilagenous mass which for a while kept the eight ascospores together.

The structure of these perithecia, formed by heterogenous hyphae, not by homogenous ones as in general in *Arachniotus* and *Amauroascus*, but circinate as in the genus *Myxotrichum* and not pectinate-dentate, nor spiral as in the genus *Ctenomyces*, permitted to put this fungus, in the genus *Gymnoascus* Baranetzky (Fam. Gymnoascaceae Baranetzky 1872; Subfam. Gymnoascaceae Castellani & Chalmers 1918; Sect. Eugymnoascaceae Nannizzi 1926). Accordingly, he renamed *Achorion gypseum* Bodin, — *Gymnoascus gypseum* (Bodin).

R. Biltris, 1929

Biltris saw by passing, in a culture of *Tr. gypseum granulosum* on 3 per cent glucose agar, organs of fructification which he considered "asci without perithecium." There is no further discussion on the subject in his paper, except for mentioning that he was unable to reproduce these "asci without perithecium" in subcultures.

M. Langeron & S. Milochevitch, 1930

Langeron & Milochevitch acknowledged that before their search for perithecium in dermatophytes investigations had been carried out by MATRUCHOT & DASSONVILLE, by CHALMERS & MARSHALL and by NANNIZZI.

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[1]) P. Tate expressed the same views in this matter. Cf.l.c., p. 70.

They recognized that *Eidamella spinosa*, MATRUCHOT & DASSONVILLE, belonged to the Gymnoascaceae. However, they expressed their doubts that this fungus had been the causative agent of ringworm in a dog, because this fungus, when reinoculated, was apathogen.

Concerning the findings of CHALMERS & MARSHALL, they believed, judging from the photomicrographs of these authors, that the perithecium depicted was the perithecium of a Perisporiaceae such as *Penicillium, Aspergillus* or *Sterigmatocystis*. They considered the fungus cultured by CHALMERS & MARSHALL to probably be contaminant. Against this assumption militates the fact that CHALMERS & MARSHALL did reproduce experimentally the same lesions with a pure culture of *Tr. currii*. This fungus, thus, was undoubtedly pathogenic. What is probable, however, is that this fungus did not belong to the Gymnoascaceae, but to the Aspergillales. There is no evidence which would prove that all the dermatophytes must belong to the Gymnoascaceae.

They also questioned the correctness of the experiments and results of NANNIZZI. They insinuated that NANNIZZI worked with impure and unsterilized material.[1]) Their assumption has been duly refuted by POLLACCI. Furthermore, they objected to the schematic drawings of the pycnidia given by NANNIZZI, and for these reasons questioned the validity of his findings.

Nonetheless, they conceded that NANNIZZI, after MATRUCHOT & DASSONVILLE, came the closest to the truth. At the end of their discussion they stated that "A fertile, complete perithecium was never yet seen in a dermatophyte in pure culture."

In contradiction to this statement, LANGERON & MILOCHEVITCH included three tables (XVII, XVIII, XIX) in their publication (lb) showing the photomicrographs of "perithecium" of *Ctenomyces granulosus*, *Ctenomyces asteroides*, and *Ctenomyces interdigitalis*. Although photomicrographs and not "schematic drawings" these illustrations are most unconvincing. There are no pycnidia, let alone perithecia, asci and/or ascospores visible in any of these illustrations. Although they concluded that *Tr. asteroides*, *granu-*

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[1]) LANGERON & MILOCHEVITCH'S (deuzième mémoire, page 503, 1930b) insinuation aimed at destroying the validity of his results. There was no foundation for this statement because NANNIZZI had clearly stated (1926, p. 88): "Dopo aver provveduto alla sterilizzazione del materiale in autoclave, insemenzai i substrati con diversi Trichophyton."

Furthermore, LANGERON & MILOCHEVITCH stated, (Note préliminaire, 1930a, p. 423—424) "TATE (1929) a reproduit les expériences de NANNIZZI mais n'a jamais vu apparaître ces organs globuleux." This statement is also without any foundation and, to say the least, is most unfair to TATE. There is not a single word or reference in the publication of TATE that he had ever duplicated NANNIZZI'S experiment.

losum, *interdigitale* and even *radiolatum* have the same morphology and thus, should be considered synonymous with *Tr. mentagrophytes* (CH. ROBIN, 1853), they seemingly intended to show three different "perithecia" in these variants of the same fungus. According to their own statement, LANGERON & MILOCHEVITCH never saw either pycnidia or perithecia in the culture of any dermatophytes on natural mediums.

CIFERRI & REDAELLI, 1947—1949

These authors tried to duplicate NANNIZZI's experiment. Their attempt to obtain pycnidic or ascus-bearing forms of dermatophytes, however, did not lead to satisfactory results. They tried to produce higher organs of fructification in *Tr. crateriforme*, *Ep. rubrum* and *A. gypseum*. They let them grow for a period of three years (!!) in darkness (underground and in cellars) on so-called natural mediums (hen feathers, horse hair, fresh horse hoofs, raw tanned leather, fresh and ripe cattle manure, dried and silo hay, garden soil) without obtaining any type of higher fructification.

It was observed, however, that most of the species used in the experiment remained viable on most of the natural mediums and there was no tendency toward pleomorphism. On the contrary, it was sometimes observed that pleomorphic strains reverted to normal morphology.

SUMMARY

Very little progress had been made on the question whether organs of higher fructification occur in dermatophytes. Only two significant studies can be noted. One was the elaboration of the hypothesis by MATRUCHOT & DASSONVILLE that "dermatophytes" because of morphological similarities with certain fungi (*Myxotrichum*, *Gymnoascus*, *Arachniotus*) belong with them to the Gymnoascaceae. However, this generalization was unwarranted, since their hypothesis was based on a single strain of a dermatophyte, designated by them as "*Trichophyton*, sp." without the species having been more closely determined. Moreover, they did not produce any evidence for their generalization that all dermatophytes should, or necessarily must belong to the Gymnoascaceae. Finally, MATRUCHOT & DASSONVILLE had never seen either pycnidia or perithecia in their cultures. To say the least, their hypothesis, whether right or wrong, must await further demonstrations that pycnidia and/or perithecia do exist in the dermatophytes.

The only apparently successful attempt at solving the question of the existence of higher organs of fructification in dermatophytes has been made by NANNIZZI. His findings must be considered valid, unless duplications of his experiments would prove to the contrary. However, the same objection has to be made against his generalization as was raised against the generalization of MATRUCHOT &

Dassonville. This is that ALL dermatophytes must necessarily belong to the Gymnoascaceae. Nannizzi succeeded in producing pycnidia only in the form-genus *Trichophyton,* in the species *Tr. mentagrophytes* (Ch. Robin, 1853) and *Tr. equinum* Matruchot. He also was successful in his endeavor in the form—genus *Achorion,* in the species *Achorion gypseum* Bodin. However, his attempts to produce higher organs of fructification in *Microsporon lanosum* Sabouraud and in *Achorion Quinckeanum* Zopf failed.

ZUSAMMENFASSUNG

Versuche, betreffs des gegenwärtigen Standes der Fruchtkörperproduktion in Dermatophyten, die sich nun auf sechs Jahrzehnte erstrecken, sind kritisch beleuchtet worden.

Es konnte nur ein einziger, anscheinend erfolgreicher Versuch verzeichnet werden, der von Nannizzi, 1926. Mit seiner speziellen Technik, indem er Dermatophyten auf "natürlichen Nährböden" (Federn, Leder, Knochen, Haare) züchtete, war es ihm gelungen, Pykniden in der Form-Gattung *Trichophyton,* in den Arten *Tr. mentagrophytes* (Ch. Robin, 1853) und *Tr. equinum* Matruchot hervorzurufen. In der Form-Gattung *Achorion* sind Pykniden in der Art *Achorion gypseum* Bodin produziert worden in einem Nährboden von Waldboden gemischt mit Stückchen von altem Leder. Jedoch sind seine Versuche, Pykniden in *Microsporon lanosum* Sabouraud und in *Achorion Quinckeanum* Zopf hervorzurufen, fehlgeschlagen.

BIBLIOGRAPHY

Biltris, R. (1929) Sur la variabilité des caractères de l'espèce chez les dermatophytes. Ann. Int. Pasteur **43:** 281—358, 15 figs.

Chalmers, A. J. & Marshall, A. (1914a) Tinea capitis tropicalis in the Anglo-Egyptian Sudan. J. Trop. Med. **17:** (17), 257—265, Sept. 1.

—, &—, (1914b) The systemic position of the genus Trichophyton Malmsten 1845. J. Trop. Med. **17:** (19), 289—291, Oct. 1.

Ciferri, R., & Redaelli, R. (1947—49) Mancata formazione di forme ascofore e conservazione di culture di funghi patogeni in substrati naturali. Mycopathologia,
131 **4:** ~~313~~—136.

Langeron, M. & Milochevitch, S. (1930a)Morphologie des dermatophytes sur milieux naturels et milieux à base de polysaccharides. (Note prèliminaire). Ann. de Dermat. et de Syph. **8:** (3—4) 422—436.

—, &—, (1930b), Morphologie des dermatophytes sur milieux naturels et milieux à base de polysaccharides. Essai de classification. (Deuzième mémoire). Ann. de Dermat. et de Syph. **8:** (5), 465—508, with 10 Tables.

Matruchot, L. & Dassonville, Ch. (1899a) Sur la position systématique des Trichophytons et des formes voisines dans la classification des champignons. C.R. Acad. Sci. **128:** 1411—1413.

—, &—, (1899b) Sur le champignon de l'herpès (Trichophyton) et les formes voisines et sur la classification des ascomycetes. Bul. Soc. Myc. France **15:** 240—253.

—, & —, (1900) Sur une forme de réproduction d'ordre élevé chez les Trichophytons. Bul. Soc. Myc. France **16:** 201—208.

—, & —, (1901a) Eidamella spinosa, dermatophyte produisant des périthèces. Bul. Soc. Myc. France **17:** 123—132.

—, & —, (1901b) Problèmes mycologiques rélatif aux teignes. Bul. Soc. Méd. Vét. **55:** 349—362.

NANNIZZI, A. (1926) Ricerche sui rapporti morfologici e biologici tra Gymnoascacee e Dermatomiceti. Annales Mycologici. Ed. by H. Sydow, **24:** 1/2, R. Friedlaender & Sohn, Berlin.

—, (1927) Ricerche sull' origine saprofitica dei funghi delle tigne. II. Gymnoascus gypseum sp. n., forma ascofora del Sabouraudites (Achorion) gypseum (Bodin) Ota et Langeron. R. Accademia dei Fisiocritici in Siena nell' Adunanza del 29 Aprile. Istituto Botanico della R. Università di Siena.

POLLACCI, G. (1931) Sulla posizione sistematica dei miceti delle tigne. (Rivendicazione). Atti dell' Istituto Botanico dell' Università di Pavia, Serie XV, Vol. III, Luglio.

TATE, P. (1929) The dermatophytes or ringworm fungi. Biol. Rev. **IV**, 41—75, VII pl.

WILENCZYK, A. (1926) Le Trichophyton en goutte pendante. Séances des 28 Octobre et 25 Novembre. C.R. Soc. Biol. **94:** 201—204.

—, (1927) Sur la formation d'asques dans le Trichophyton. (deuxième mémoire). Ann. Inst. Pasteur **41:** (12): 1351—1356.

—, (1928) Formation des asques dans les cultures de Trichophyton. C.R. Soc. Biol. **98**(1): 70.

FUNGI OCCURRING ON PINUS IN PAKISTAN

by

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(24.VI.1960)

The genus *Pinus* in Pakistan is represented by three native species i.e., *Pinus longifolia* (*P. roxburghii*), *Pinus excelsa* (*P. wallichiana*) and *Pinus gerardiana*. The first two are timber species and the last one is primarily valued for its edible seeds.

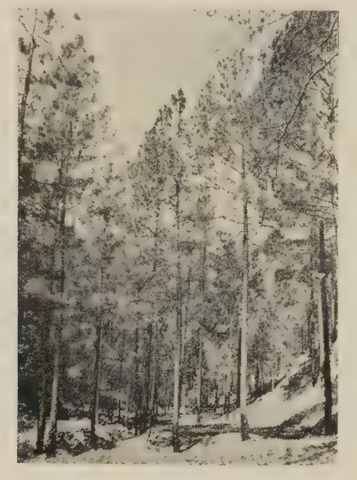

Fig. 1

Pinus longifolia (*Chir*, longleaf pine) is very common in lower hills from 1500 ft. to 5,000 ft. It is a large evergreen tree with somewhat whorled branches forming a pyramidical crown which later becomes umbrella shaped (Fig. 1). The heartwood is moderately hard and reddish brown. This pine grows up to 150 ft. in height with 7—14 ft. girth. Under unfavourable conditions it may be stunted. It is sometimes planted in the gardens in the northern plains of West Pakistan. This tree due to its easy accessibility is subject to much misuse. It is the main source of resin in Pakistan, besides its timber. The living tree and its timber are subject to fungus attack and various species have been enumerated in this paper.

Pinus excelsa: (Blue pine, *Kail*) is an important timber species of West Pakistan and ranges next to *deodar* in its use in construction work (Fig. 2). It has blue green foliage. Branches are whorled, spreading, usually ascending in young trees, horizontal to drooping in old ones. The heartwood is moderately hard, pink, of good quality and easily worked. It also yields resin of very good quality but yield is much less than *Pinus longifolia*. The trees become bent near the base when young plants are pressed by the sliding snow.

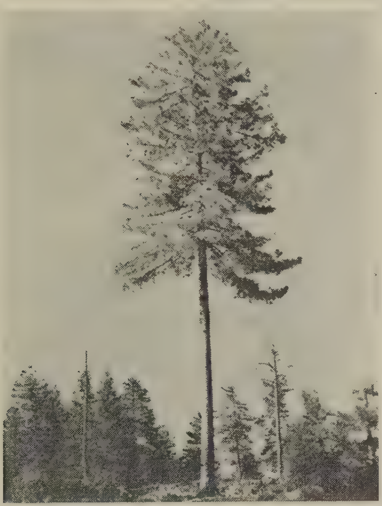
Fig. 2

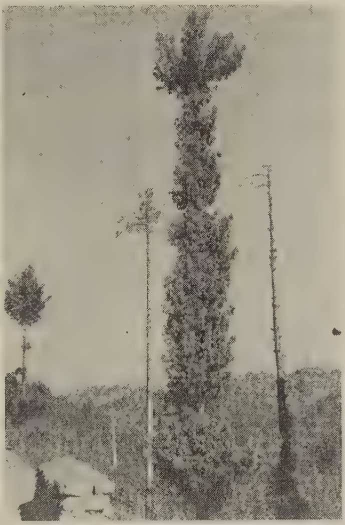
Fig. 3

It reaches a height of 165 ft. and girth of 9 ft., average is about 118 ft. and over eight ft. in girth. In its prime it is one of the most graceful pines. The blue pine is found chiefly from 6,000 ft. to 10,000 ft. It descends to 4,000 ft. on northern aspects in some localities where it gets mixed up with *Pinus longifolia*. It occurs both pure and in mixed formations. Blue pine is subject to various types of injuries.

Kail suffers from extensive lopping in private forests and ultimately dies, although it stubbornly tries to throw out adventitious branches, first towards sunny sides. (Fig. 3). Severe damage is done in private forest areas.

Pinus excelsa (*P. wallichiana*) suffers from snow more than any other conifer. The snow settles on the crown and makes it top heavy and the tree either breaks from the top or falls down.

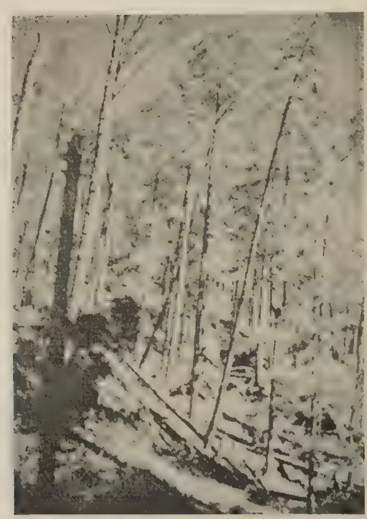

Fig. 4

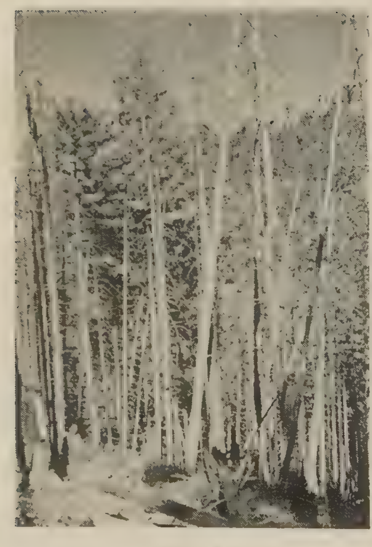

Fig. 5

Fig. 6

Recently there has been seen extensive damage due to snow followed by slight wind in Azad Kashmir (Fig. 4).

Fire does extensive damage sometimes as seen in fig. 5. Many trees die as a result of this injury.

Besides damage by physical agencies, the tree also shows some-

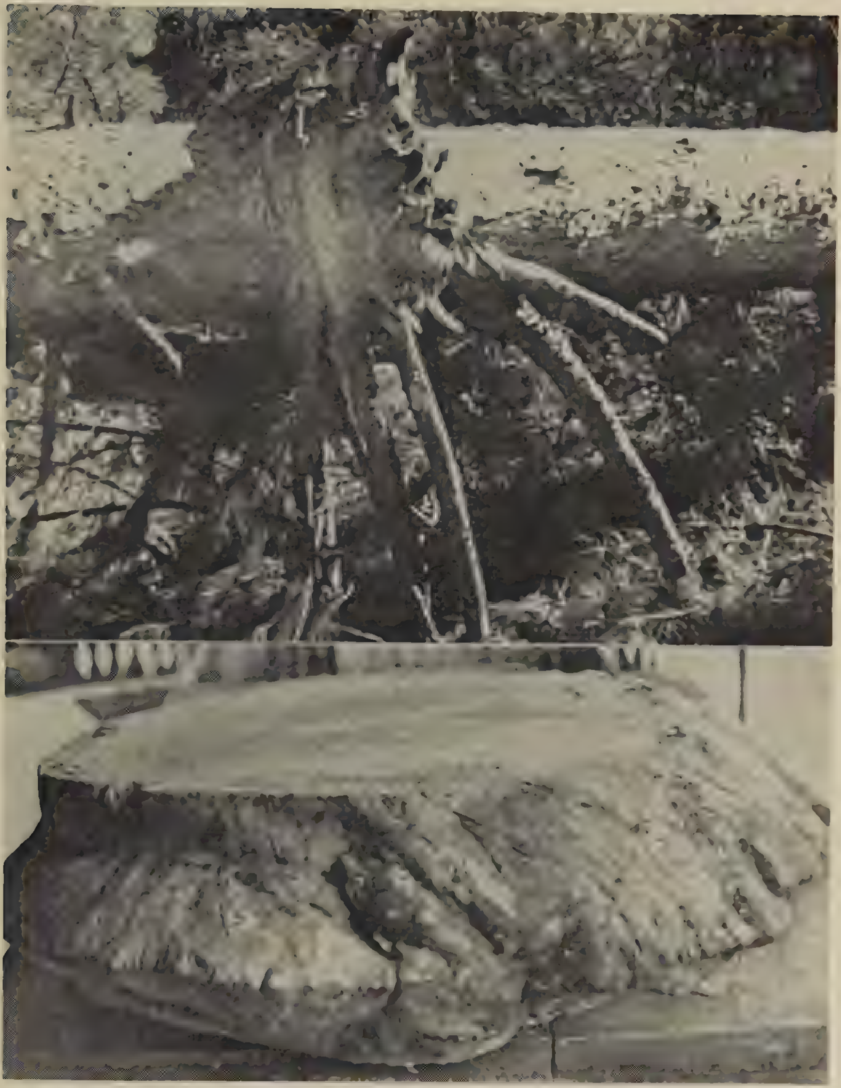

Fig. 6a, 6b.

times burls and gnaurs (Fig. 6, 6a, 6b). This is believed to be due to woodpeckers who peck and girdle around the tree in search of insects. The injury results in stimulation and callus formation ending up as a swelling around. The adventitious shoots coming out get glued with the resin oozing out and often one finds small branches forming a half circle along the burl.

Another malformation that has frequently been noted in blue

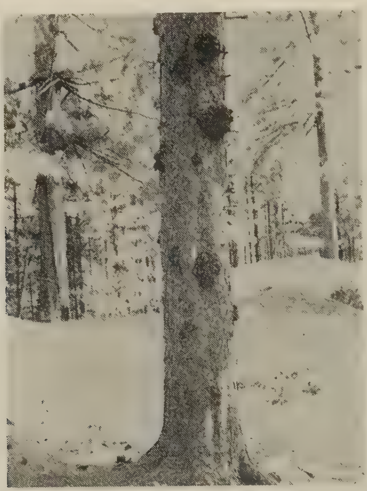

Fig. 7

pine in Azad Kashmir is the formation of large clinkers which burst outwards (Fig. 7, 7a). The tree is virtually studded and is rendered useless. A small streak of rot is commonly associated with the clinker. Work on the causal agency is in progress. A number of pathogens including some well known pathogens are recorded in this communication.

The *Chilghoza pine* (*Pinus gerardiana*) is a moderate sized, much branched tree about 50 ft. to 60 ft. high and 6—8 ft. in girth. Branches are ascending but not whorled. Needles are in threes, like *Pinus longifolia*, but not so long, shorter and stiffer. Wood is tough, resinous and not much used except in scarcity. The tree is valued for its seed and grows at 7,000 ft. to 11,000 ft., in the arid inner valleys subject to winter snow. No work has been done on the fungal pathogens of this species.

The fungi growing on *Pinus* species are briefly discussed in the following pages.

I. Ascomycetes:

1. *Hysterium macrospermum* Peck.
   (Hysteriaceae).
   This fungus has been recorded on dead wood of *Pinus excelsa*, from Charehan, (Murree hills) by Ahmad (1956).
2. *Podocrea cordicens* Penz. & Saac;
   (Hypocreales)
   This fungus has been reported by Ahmad (1956) on rotten cones of *Pinus excelsa*. No significant damage has been observed in forests.

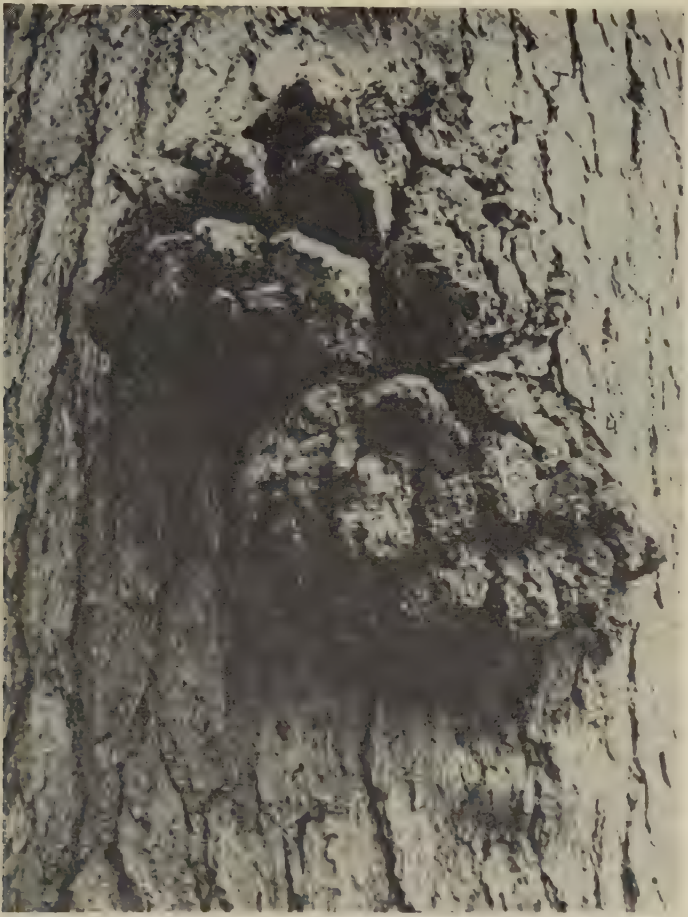

Fig. 7a

3. *Schizoxylon insigne* (DE NOT). REHM & RABB; Syn. *Oomyces insignis* (DE NOT). *Schizoxylon alboatrum* REHM. *S. occidentale* ELL. & FR.

   Reported by AHMAD (1956) on decorticated branches of *Pinus excelsa*, Murree (Charehan). It is a saprophyte on dead wood.

4. *Lophodermium pini-excelsae* AHMAD.
   (Phacidaceae).

   This causes needle-cast in moist and shady localities. It has been collected on leaves of *Pinus excelsa*, from Kaghan valley, Shogran, Murree hills and Azad Kashmir. The damage is rarely serious as to need special control measures.

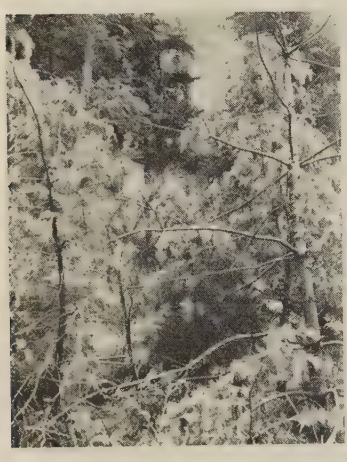

Fig. 8

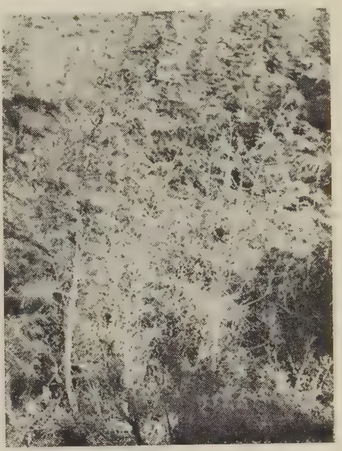

Fig. 9

5. *Therrya cembrae* (Rehm. Cash). Cash. Comb. nov.
   Reported by Ahmad (1956) on dead branches of *Pinus excelsa*, in Kaghan valley, Shogran, Swat.
6. *Dascypha fusco-sanguinea* Rehm. emend. Hoehnel.
   This fungus kills sporadically young plants of *Pinus excelsa* in Kaghan valley (Naran). Further work on Pathology of this fungus is in progress. (Fig. 8).
7. *Cenangium ferruginosum* (Fr.) Fr. (= *C. abietis* by Seavers).
   This fungus also kills the young *Pinus excelsa* plants about 10 years old in moist localities. Further work on this species is in progress. (Fig. 9).

II. Basidiomycetes:

8. *Cronartium himalayense* Bagchee.
   (Uredinales).
   This fungus is a serious rust of *Pinus longifolia* in India where there are more rains than in Pakistan. Here this fungus is rare, although the alternate host, the *Swertia* species occur here also. This is perhaps due to drier conditions prevailing in Pakistan (c.f. Bagchee 1929, 1940).
9. *Cronartium ribicola* Fischer (= *Peridermium indicum* Colley & Taylor).
   This is serious stem rust of *Pinus excelsa*. Although it has been mentioned by Hafiz Khan (1928) and Malik (1944), yet it is rare as far as the experience of the author is concerned. Its biology and pathology has been exhaustively dealt with by Bagchee (1950). It has also been collected by Ahmad (1956). from Shogran.

10. *Peridermium brevius* (BARCL.) SACC. (= *Coleosporium barclayense*).

This needle rust is found in Murree hills on *Pinus excelsa*. The damage is not serious. The fungus causes early shedding of needles. (cf. BAGCHEE 1950).

11. *Peridermium orientale* CKE. (= *Coleosporium campanulae* PERS. LEV.; *P. complanatum* Var. *Acicola* BARCL.)

This is a needle rust of chir pine and is somewhat serious in Siran Forest Division in Daddar and Batrassi as well as in Junjal Hill in Azad Kashmir. It causes early shedding of needles. (cf. BAGCHEE 1941).

12. *Hymenochaete leonina* BERK. & CURT.
(Thelephoraceae).

It has been reported by AHMAD (1956) on stumps of *Pinus excelsa* in Murree hills.

13. *Peniophora gigantea* (FR.) MASSEE, LINN.

It has been reported by AHMAD (1956) on dead wood of *Pinus excelsa* and *Abies pindrow* in Murree (Patriata) and Shogran.

14. *Stereum chailletti* (PERS. ex. FR.). FR., *Lloydella chaillettii* (PERS. ex. FR.). BRES.

It has been reported by AHMAD (1956) on stump of *Pinus excelsa* Murree.

15. *Stereum purpureum* (PERS. ex. FR.) FR.
(Thelephoraceae).

This fungus has been collected by AHMAD (1956) from (Charehan) Murree hills, on *Pinus excelsa*.

16. *Stereum fascum* (SCHRADER) QUEL.

It has been collected on *Q. incana* and *Pinus excelsa* by AHMAD (1956).

17. *Auriscalpium vulgare* S. F. GRAY — Syn. *Hydnum auriscalpium* (LINN. ex. FR.). *Pleurodon aurriscalpium* (LINN. ex. FR.). PAT.
(Hydnaceae).

It has been recorded by AHMAD (1956) on decayed cones of *Pinus excelsa*, in Patriata and Murree hills.

18. *Odontia arguta* (FR.). QUEL.
(Hydnaceae).

On stump of *Pinus excelsa* (AHMAD 1956).

19. *Merulius aureus* FR. SACC.
(Meruliaceae).

It has been collected by S. AHMAD (1956), on logs of *Pinus excelsa* in Kaghan valley, Shogran. It causes white spongy rot in wood in moist localities. (Fig. 10).

20. *Merulius tremellosus* SCHRAD.

It has been reported on coniferous logs from Charehan and Nathiagali (Murree hills) by AHMAD (1956).

21. *Fomes annosus* FR. CKE.
(Polyporaceae).

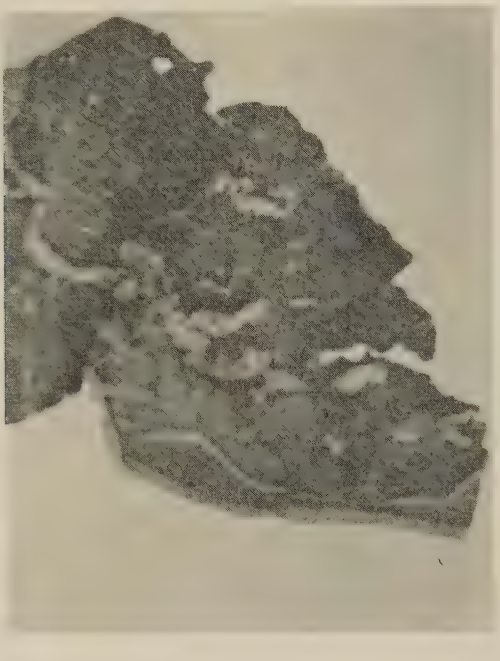

Fig. 10

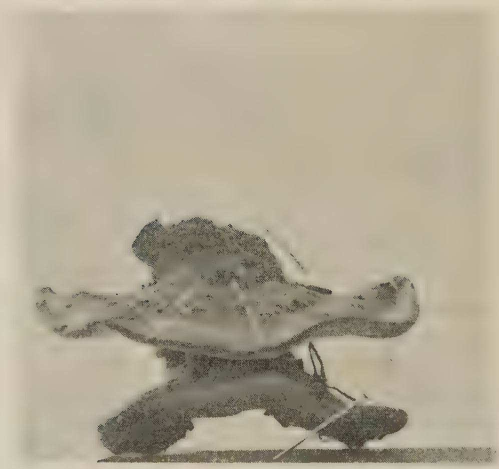

Fig. 11

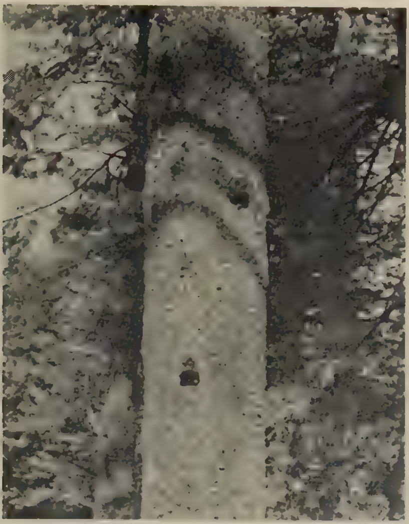

Fig. 12

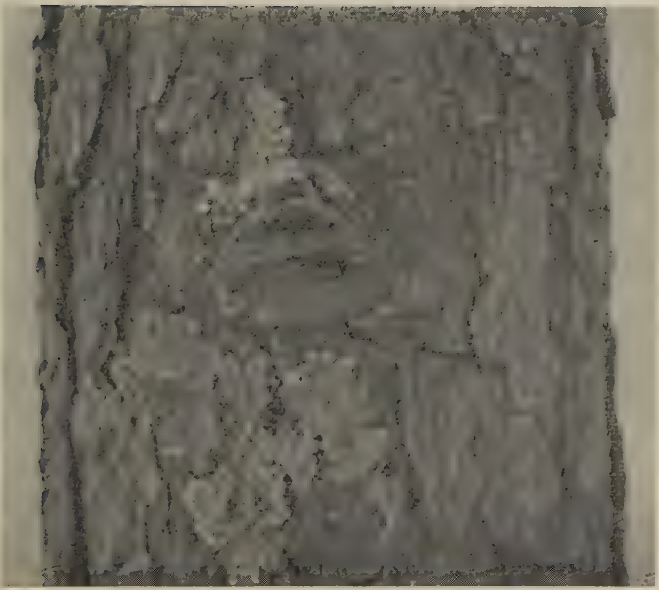

Fig. 12a

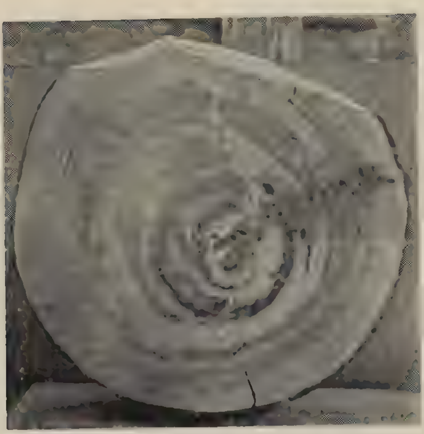

Fig. 12b

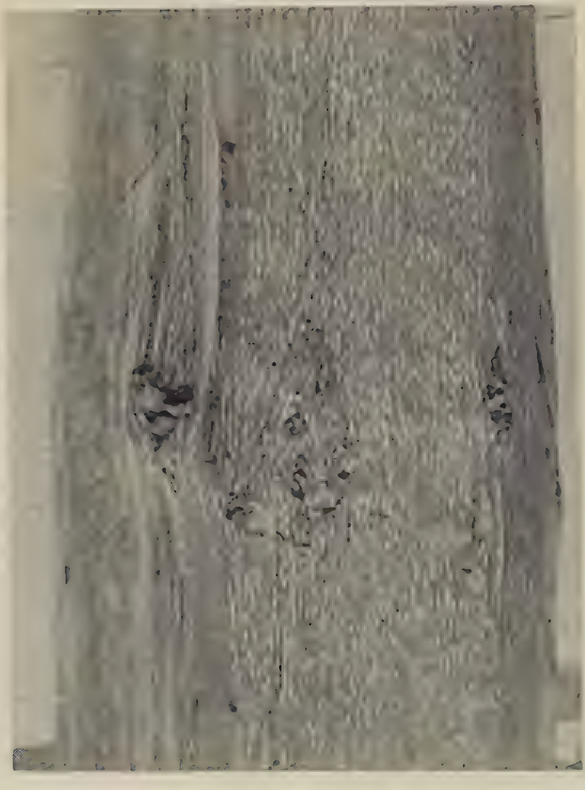

Fig. 12c

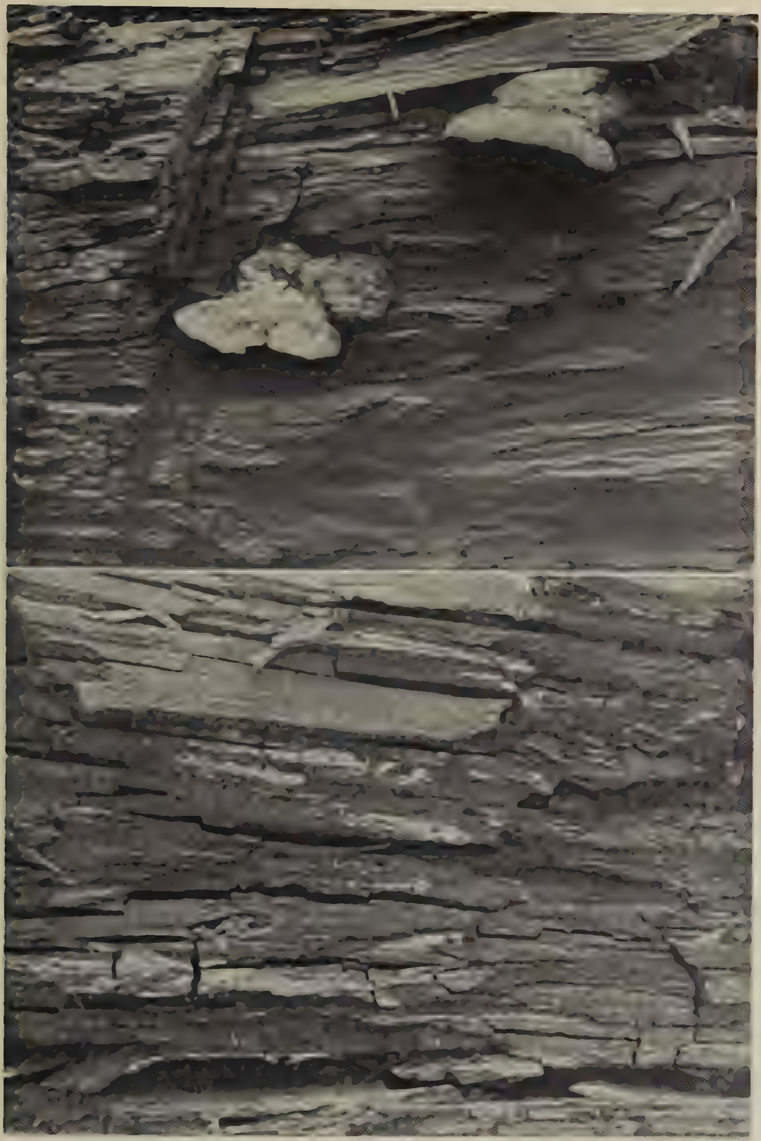

Fig. 13, 13a

This is a common and widely known fungus on conifers. The damage is not of great significance except in very moist localities and once established causes a serious root rot in conifers. It grows as a saprophyte on stumps. Pathology of this fungus is too well known to be repeated here. It has been seen on roots of *Pinus excelsa,* in Trarkhel, Azad Kashmir and on stump of *Pinus longifolia* in Murree hills. (Fig. 11).

22. *Fomes borneonensis* (LLOYD n. comb). Syn. *Trametes borneonensis* LLOYD.

It has been recorded by AHMAD (1956) on *Pinus excelsa,* KAGHAN valley, (SHOGRAN) Swat, (Kalam).

23. *Fomes pectinatus* KL.
(Polyporaceae).

It occurs occasionally on *Pinus excelsa,* in Junjal hills, (Azad Kashmir) where it causes brownrot in wood. In hardwoods it is noted to cause white rot.

24. *Fomes pini* (THORE. ex. FR.) KARST.

This fungus is world wide in distribution and is a serious pathogen of conifers especially *Pinus excelsa* in West Pakistan. It has been collected from living trees of *P. excelsa,* Murree, (Charehan, Ghora gali), Swat, Kaghan valley, (Shogran, Shoran); Azad Kashmir, etc. The fungus causes characteristic white pockets in decaying timber. Various stages of rot are given in the photographs (Fig. 12, 12a, 12b, & 12c). (cf. BUTLER, 1905, COOKE, 1876). It has also been noted sometimes on *Pinus longifolia.*

25. Var. *abietis* f. *micropora* PILAT;

It has been collected on *P. longifolia* from Azad Kashmir; on stump of *P. excelsa,* Patriata. (AHMAD 1956).

26. *Fomes pinicola* SWARTZ. ex. FR.

It occurs mainly on dead wood but is a wound parasite found growing on otherwise weak trees. It has been seen on living trees of *P. excelsa* in Nathiagali, Kaghan valley, (Shogran); Azad Kashmir and causes brown cubical rot. It spreads quickly and in a couple of years the wood may be a crumbly heap of friable mass. (Fig. 13, 13a).

27. *Fomes robustus* KARST.

It has been recorded by AHMAD (1956) on conifer wood from Kaghan valley, (Shogran).

28. *Fomes roseus* (A & S). FR.

It is occasionally found on dead coniferous wood causing brown cubical rot.

29. *Ganoderma applanatum* (PERS.) PAT.
(Polyporaceae).

This fungus is not infrequent at higher altitudes and mostly grows on stumps and dry standing trees. It has been collected from Trarkhal, in Azad Kashmir and causes white rot.

30. *Ganoderma lucidum* (Leys. Fr.). Karst.

The fungus has a wide host range and has been seen on dead roots of conifers in A. Kashmir. Mostly it is found in the forest debris in temperate areas. It is quite common.

31. *Lenzites betulina* Linn. ex. Fr.

(Polyporaceae).

It is sometimes found on fallen logs of *P. excelsa* in Murree hills (Patriata). It causes white spongy rot, but is infrequent on conifers.

32. *Lenzites sepiaria* (Wulf.) Fr.

This is not so common as *L. subferruginea* but is equally destructive. It attacks coniferous wood and has been collected on *P. excelsa* from Junjal Hill.

33. *Lenzites subferruginea* Berk.

This is a very common species occurring mostly on coniferous timber. It attacks frequently felled timber in the forest and

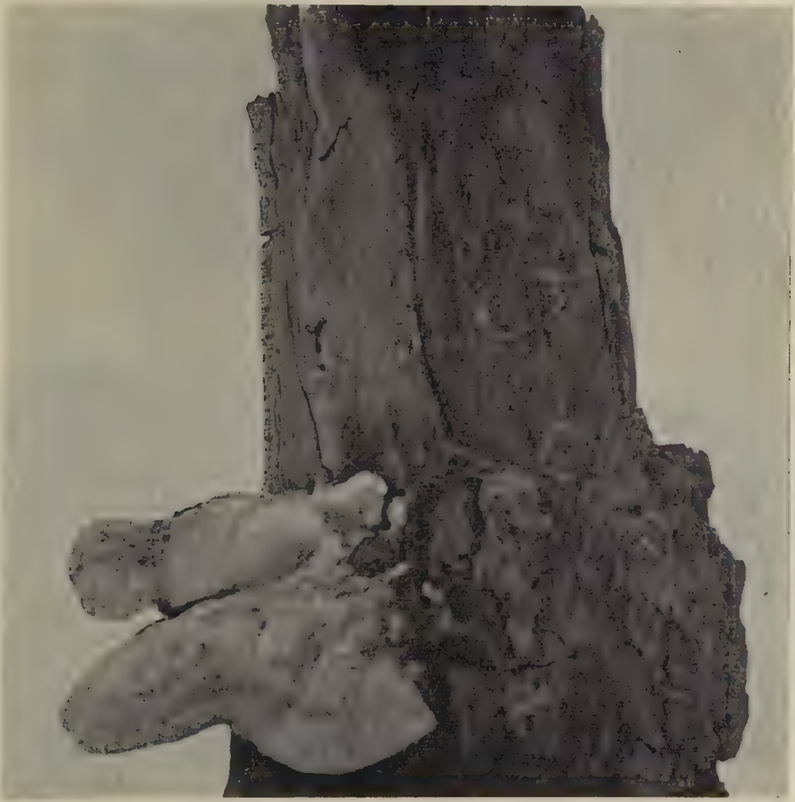

Fig. 14

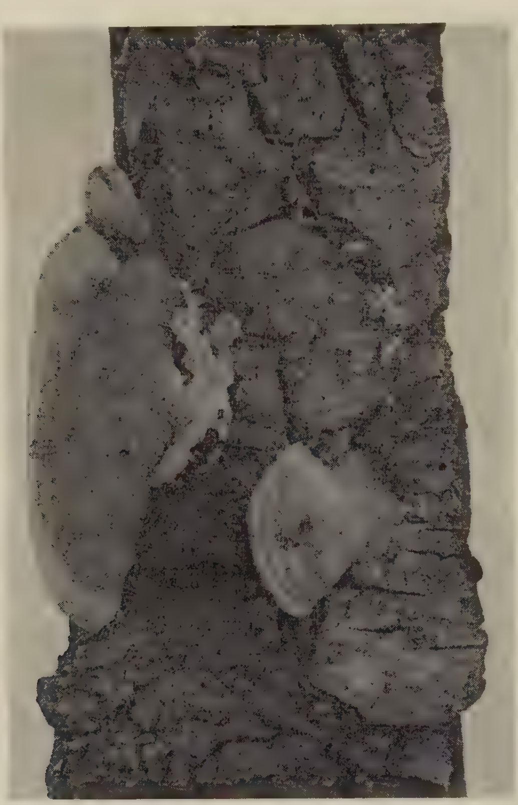

Fig. 14a

timber depots, and causes brown cubical rot. This species is a serious wood rot which destroys timber in a few years. It can stand quite a bit of desiccation. The fungus has been collected from temperate regions all over West Pakistan, especially on *Pinus excelsa* and *Pinus longifolia.* (Fig. 14, 14a).

34. *Lenzites striata* Swartz. ex. Fr.

This species produces brown cubical rot in coniferous timber in Kaghan, Murree hills and A. Kashmir. It has also been collected on *Pinus longifolia* in Kund area.

35. *Lenzites* sp.

It is a yellow lenzites with dark red gills. It is uncommon and has been collected on *Pinus longifolia* stumps in Azad Kashmir.

36. *Polyporus dryaedeus* Pers. ex. Fr.
(Polyporaceae).

It has been collected by Ahmad (1956) on a stump of *Pinus excelsa,* Murree.

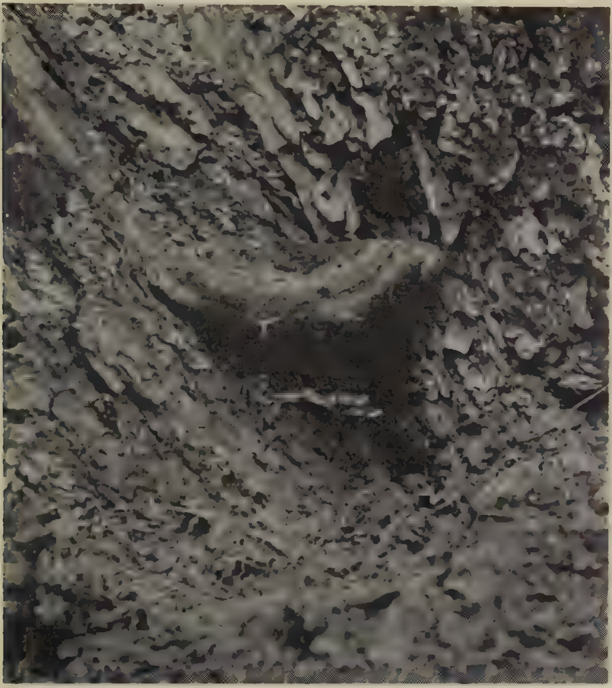

Fig. 15

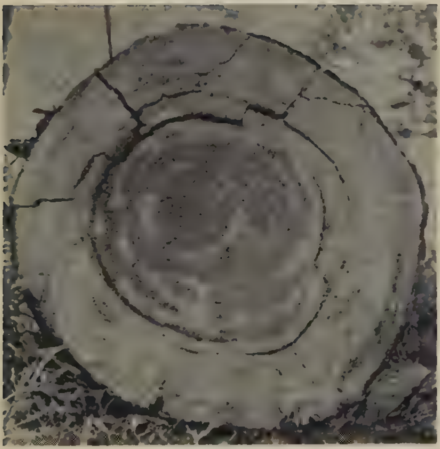

Fig. 15a

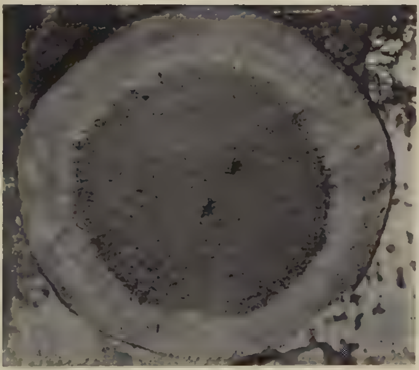

Fig. 15b

37. *Polyporus gilvus* (Schw). Fr.

This is more common on hardwoods but occasionally is seen attacking conifers. It has been collected on roots of *Pinus excelsa,* in Trarkhel, Azad Kashmir.

38. *Polyporus ostreiformis* Berk.

It has been collected on *Pinus excelsa* causing white rot.

39. *Polyporus schweinitzii* Fr.

(Polyporaceae).

It is a serious butt rot of conifers especially on *Pinus excelsa* and is likely to be confused with the rot due to *Fomes pini.* It causes brown cubical rot. Boreholes are small and spiral cracks in the tracheids are seen. Old hyphae are constricted at boreholes. (Fig. 15, 15a, 15b).

40. *Polyporus sulphureus* Bull. ex. Fr.

It is not so common in our forests. The fruit bodies are strikingly orange yellow above and yellow below. It causes brown cubical rot and has been collected growing on pole of *Pinus longifolia* in Kund area (Siran Forest Division, Hazara.)

41. *Polyporus* sp.

It is an interesting species causing brown rot.

42. *Polystictus abietinus* Dicks. ex. Fr.

(Polyporaceae).

It has been collected on *Pinus excelsa* from Azad Kashmir (Garala). Also recorded by Ahmad (1956) from Swat, Murree hills, and Gallis. This fungus is found on fallen logs and timber in forests. The damage is insignificant. It causes white spongy rot.

43. *P. hirsutus* Fr.

This is a typical saprophyte on a large variety of hosts. It causes white spongy rot and has been collected on *P. longifolia,* in Poonch, Hazara and Murree hills.

44. *Polystictus tomentosus.*

It is occasionally seen on dead wood lying in the forest or at the base of trees. It causes white spongy rot on *Pinus excelsa.*

45. *P. versicolor* Linn. ex. Fr.

It causes white spongy rot on both conifers and hardwoods. On the latter it is more frequent. It has been collected on *P. excelsa,* Trarkhel (Azad Kashmir).

46. *Poria* sp.

(Polyporaceae).

It is a crimson brown poria causing spongy white rot on *Pinus excelsa,* in Trarkhel, Azad Kashmir.

47. *Trametes lactinea* Berk.

(Polyporaceae).

It occurs on fallen wood, causing white spongy rot and has been collected on logs of *Pinus longifolia.*

48. *Trametes persooni* Fr.

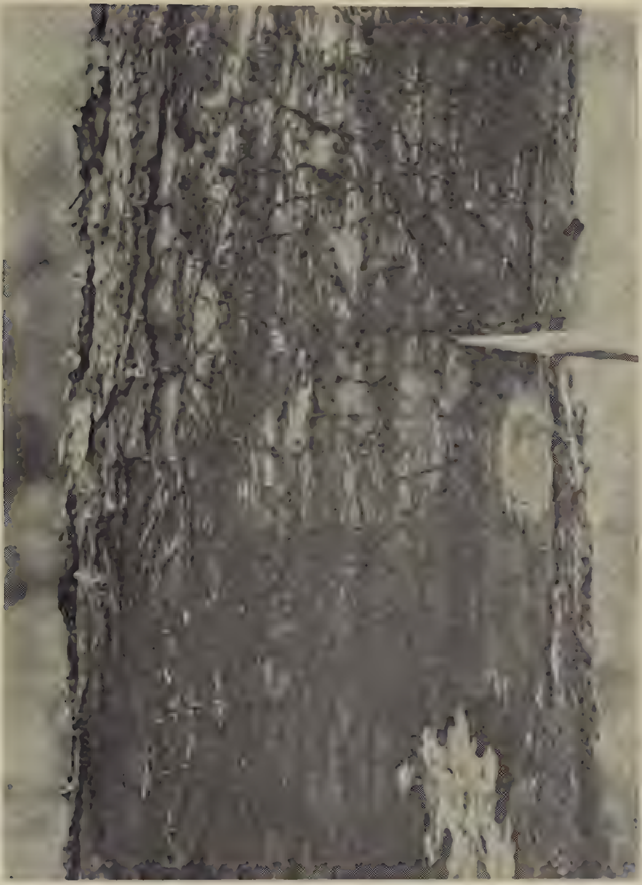

Fig. 16

The fungus has been collected from burnt log of *P. excelsa* from Trarkhel and on stumps in Murree hills.

49. *Trametes dickensii* Berk.

It causes brown cubical rot on *Pinus excelsa* wood.

50. *Armillaria mellea* (Vahl). Quel.
(Agaricaceae).

This fungus is notorious for its world wide distribution on a large number of hosts. It has been collected from Azad Kashmir on *Abies pindrow* and *Pinus excelsa* where it causes serious root rot. The rot is white stringy type with zone lines. Formation of rhizomorphs is characteristic. (Fig. 16).

51. *Schizophyllum commune* Fr.
(Agaricaceae).

It is a common saprophyte both on conifers and hardwoods, wherein it causes white rot. It has been collected on wood of *P. longifolia*, from Azad Kashmir.

III. Deuteromycetes.

52. *Leptostroma ahmadii* Petr.
(Laptostromeceae).
It has been noted on *Pinus excelsa*. Recorded by Ahmad (1956) on living and dead needles of *P. excelsa.* in Kaghan valley (Shogran).

Acknowledgement

The writer gratefully acknowledges the help and facilities provided by Dr. S. Ahmad in his herbarium.

References

1. Ahmad, S. (1956) Fungi of West Pakistan. Biol. Soc. Pak. Lahore; Monogr. 1.
2. —, (1954) Beiträge zu Pilzflora Pakistans. Sydowia, Ann. Myc. Ser. **II. 8,** 1—6.
3. —, (1956) Uredinales of West Pakistan. Biol. Pak. **2,** 1, 26—101.
4. Bagchee, K. (1941) Contributions to our knowledge of the Morphology, Cytology and Biology of Indian Coniferous rusts. Pt. I. *Cronartium Himalayense* and *Peridermium orientale* on *Pinus longifolia.* Indian For. Rec. (N.S.) Bot. **I,** (7), 247—266.
5. —, (1950) Contributions to our knowledge of the Morphology, Cytology and Biology of Indian coniferous rusts. Pt. II, Indian For. Rec. (N.S.) Bot. **4** (1).
6. Bagchee, K. (1950) Contributions to our knowledge of the Morphology, Cytology and Biology of Indian Coniferous rusts Pt. III. The biology of *P. brevius.*
7. —, (1929) Investigations on the infestations of *Peridermium complanatum* Barcl. on the needles and of *P. himalayense* n. sp. on *Pinus longifolia.* Indian For. Rec. (old sr.) Bot. **14,** (3) p. 79.
8. Butler, E. J. (1905) Some Indian Forest Fungi. Indian For. **31,** 487, 548, 544 and 611.
9. Cooke, M. C. (1876) Fungoid diseases of Forest trees. Indian For. **2,** 380.
10. Hafiz Khan, A. (1928) Preliminary report on the Peridermiums of India and the occurrence of *Cronartium ribicola* on *Ribes rubrum.* Ind. For. **54,** 8, 431.
11. Malik, S. A. & Khan, M. A. (1944) Parasitic fungi of North West Frontier Province. Indian J. agric. Sci. **13,** 522—527.

RIFOMYCIN. XI. TAXONOMIC STUDY ON STREPTOMYCES MEDITERRANEI NOV. SP.

by

P. Margalith & G. Beretta

(*Research Laboratories, Lepetit S.p.A., Milan, Italy*)

(7.VII.1960)

During the course of a screening program for new antibiotics, we came across a *Streptomyces* that was isolated from a soil sample from a pine arboretrum at an altitude of ca. 200 m and about 50 m off the sea shore of St. Raphael (France). Isolation was performed by plating the soil suspension on Benett's agar. The particular morphological appearance of the strain (receiving our collection number ME/83) as well as the antibiotic activity against Gram-positive bacteria found in shake flask broth called our attention for further work on this *Streptomyces*, which appears to be a new species called by us *Streptomyces mediterranei*.

From the fermentation broths of this *Streptomyces* a mixture of several antibiotics (rifomycin-complex) was isolated(1). One of the components of rifomycin-complex, rifomycin B, has promising therapeutical applications (2, 3, 4, 5, 6).

In this paper we would like to describe some of the morphological and biochemical characters of *Streptomyces mediterranei*.

TAXONOMY

Culture ME/83 was compared with other species of *Streptomyces* as described by Waksman & Lechevalier (7) and others. A detailed account of the morphology is given in the following description of ME/83 grown on various media, in slant cultures, for up to 20 days at 28° C. Unless stated otherwise, culture media were prepared according to Waksman(8). Color determinations were performed according to Maerz & Paul(9).

Oatmeal agar:	Fair growth with smooth surface. Vegetative mycelium hyaline to yellowish with pinkish reverse. Whitish serial mycelium with pink tinge. Traces of yellowish soluble pigment.

Yeast-extract glucose agar:	Abundant growth yellowish to pink with rough surface. Scanty aerial mycelium. No pigmentation of media.
Emerson glucose agar:	Abundant growth, yellowish to pink orange with rough surface. Aerial mycelium becomes pinkish (2/B/8). Pale amber soluble pigment
Oatmeal mix agar (1):	Good growth, smooth, yellowish with light orange tinge. Aerial mycelium whitish to pink. Amber soluble pigment.
Benett's agar:	Good growth, yellowish turning orange yellow. Aerial mycelium becoming pinkish. Light amber pigment.
Penassay agar (Difco):	Poor growth.
Yeast extract molasse agar 2):	Abundant, rough growth, colorless to yellowish. Whitish aerial mycelium. Deep amber soluble pigment (12/H/10).
Czapek-Dox sucrose agar:	Poor growth, thin and colorless to light melon (11/A/8). Traces of pinkish white aerial mycelium. No soluble pigment.
Potato agar:	Poor growth thin and colorless. Traces of whitish aerial mycelium. No soluble pigment.
Potato wedge:	Poor development, hyaline. Color of wedge not changed.
Glucose-asparagine agar:	Fair growth with smooth surface. Thin vegetative mycelium of light orange pink color (10/B/12) and yellowish reverse. No aerial mycelium. Some light yellow soluble pigment.
Glycerol-asparagine agar:	As glucose-asparagine agar.
Nutrient agar:	Moderate growth with smooth surface; melon to orange (2/B/10) with yellowish orange reverse. Aerial mycelium pinkish white. Soluble pigment absent.

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1) Oatmeal-mix agar contained in addition to oatmeal (20 g cooked out flakes filtrate): Enzymatic casein hydrolyzate 5 g; Beef extract 3 g; Sucrose 10 g; Agar agar 40 g in 1000 ml H_2O dist. pH corrected to 6.9.

2) Yeast extract molasse agar contained: Glycerol 7.5 g; Molasse 7.5 g; Yeast extract 5 g; NaCl 4 g; Agar agar 30 g; H_2O dist. 1000 ml; pH corrected to 6.0.

Pridham's agar:	Moderate growth, smooth, colorless with lobster red (2/G/11) spots. Pink aerial mycelium. No pigmentation of medium.
Starch agar:	Poor growth, colorless to light orange pink. Starch hydrolysis doubtful. Scarce white aerial mycelium.
Dextrose-Tryptone agar:	Abundant growth, orange pink with golden yellow to orange reverse. Pinkish aerial mycelium. Light golden yellow (9/J/6) soluble pigment.
Hickey's and Tresner's cobalt agar:	Moderate growth, hyaline to light pinkish orange. Some pinkish aerial mycelium. Some yellowish soluble pigment.
Tyrosine agar:	Poor growth.
Ca-malate agar:	Fair growth, colorless. Aerial mycelium whitish with pink tinge. No soluble pigment. Partial digestion of Ca-malate.
Dorset's albumin agar:	Fair growth, pink. No aerial mycelium or pigmentation of medium.
Gelatine:	No pigmentation. Liquefaction slow and uncomplete.
Nitrates:	Surface growth with pinkish aerial mycelium. No reduction to nitrites. Broth becomes yellowish.
Litmus milk:	No peptonization or coagulation. Slight alkaline reaction.
Cellulose:	No growth on filter paper disks placed on carbohydrate free mineral base agar.
Temperature:	Optimum 28° C. At 37° C, growth takes place but characters are less distinct.
Morphology of colonies:	In Benett's agar, this strain forms small (usually not more than 2 mm) colonies with well defined contours, compact but rough surface; covered with whitish aerial mycelium. A great number of colonies plated on agar do not develop aerial mycelium and remain in their orange-red vegetative mycelium that grows to considerable thickness until they assume a rock-like appearance.

Morphology of aerial mycelium

When sporulation takes place, spores appear in long chains, somewhat twisted; spirals are not visible. Spores are ellipsoidal to oblong ($0.8 — 1{,}0\,\mu \times 3.0 — 5.0\,\mu$). Fig. 1 presents the aerial mycelium of ME/83 when grown on cellophane squares placed upon inositol-base medium (see below) for 4 days at 28° C.

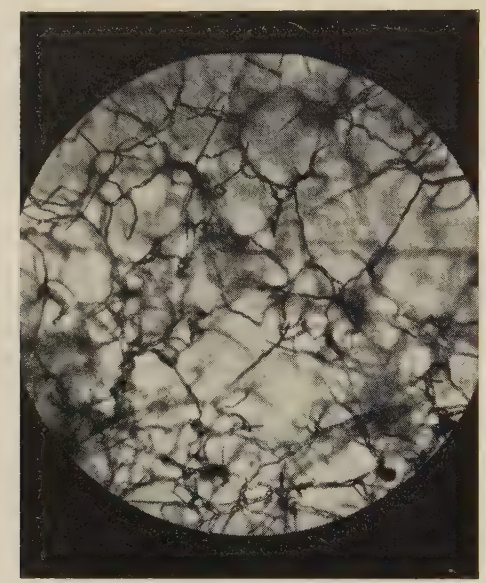

Fig. 1. Aerial mycelium of *S. mediterranei* grown on inositol-base medium (× 1000)

Test for utilization of carbon sources

These were performed according to Pridham & Gottlieb (11). Results are given in table I. It can be seen that of all the compounds tested: raffinose, inulin, sorbitol, dulcitol, citrate, acetate and glycine were not utilized by *S. mediterranei*.

Classification

Streptomycetes with similar characters have been named: *S. roseoflavus* (12), *S. microflavus* (12), *S. aurantiacus* (7) and *S. parvus* (7). Table I compares these strains with *Streptomyces* ME/83. It has to be pointed out, however, that *S. microflavus*, Waksman strain obtained from C.B.S., does not correspond to the description given by Tadashi Arai (12) in comparing the flavomycin producing strains. Also the original description given by Krassilnikov for *S. aurantiacus* as brought in Waksman (7) is insufficient and an attempt is made herewith to complete it, employing a similar strain in our laboratory.

TABLE I.

Assimilation of Carbon Compounds by S. mediterranei and similar strains on solid media 1).

Carbon source	*S. roseoflavus* 2)	*S. microflavus* 3)	*S. mediterranei*	*S. parvus*	*S. aurantiacus*
Arabinose	+	—	+++	N.D. 4)	±
Rhamnose	—	++	++		—
Xylose	±	+	++		—
Glucose	+++	++	++		—
Galactose	—	++	+++		++
Fructose	—	+	+++		—
Mannose	—	++	++		+
Lactose	—	—	++		—
Maltose	+++	++	+		—
Sucrose	—	—	++		—
Raffinose	—	—	—		—
Dextrin	++				
Inulin	—	—	—		—
Dulcitol	—	—	—		—
Glicerol	+++	++	+++		—
Mannitol	—	++	++		±
Inositol	—	—	++		—
Sorbitol	—	—	—		—
Na succinate	N.D.	++	+		—
Na citrate		++	—		—
Na acetate		+	—		—
Glycine		—	—		±
Salicin		—	+		—

1) Degrees of growth after 5 days incubation are indicated by +, ++ or +++. No growth is indicated by —.

2) Description from (12).

3) WAKSMAN strain obtained from C.B.S.

4) No data available.

The most important characters of S. ME/83 can be summarized as follows:

a) It does not belong to the chromogenic group.
b) On most media vegetative growth assumes a yellowish to orange color, but never an intense orange red to cherry or lavender intonation.
c) Poor sporulation on most media.
d) Characteristic utilization of carbon sources.

Since streptomycetes similar to ME/83 are different in many major characteristics, we feel justified in naming this isolate *S. mediterranei* nov. sp. Most probably this species should be grouped together with the former ones in a *S. roseus* series or group, though not in the sense of WAKSMAN (7) where also species like *S. fradiae* are included.

TABLE II.
Comparison of ME/83 with other similar strains

	Streptomyces 1) *roseoflavus*	*Streptomyces* 2) *microflavus*	*Streptomyces ME/83*	*Streptomyces* 3) *parvus*	*Streptomyces* 4) *aurantiacus*
Spores	oval to oblong	ellipsoidal to rodlike	rodlike	spherical to ellipsoidal	non visible
Vegetative mycelium	yellow to orange	hyaline	yellowish to pink orange	golden yellow to brick-red	orange or orange brown
Aerial mycelium	pinkish white, spirals present, sporophores with spores in chains	velvety, white, spirals and sporophores not visible	mealy, pinkish white, spirals not visible, sporophores with spores in chains	rose-white spirals and sporophores present	scarce, carnation-red or pinkish orange
Soluble pigment	absent	mostly absent, on some media violet pigmentation	absent or yellowish	N.D. 5)	absent or amber
Nitrates	reduced	not reduced	not reduced	slightly reduced	not reduced
Gelatine	strong liquefaction, orange-brown pigment	strong liquefaction, no pigment	little to no liquefaction, no pigment	slow liquefaction, no pigment	liquefaction, no pigment
Cellulose	decomposition	no decomposition	no decomposition	N.D.	no decomposition
Litmus milk	coagulation and peptonization, alkaline reaction	strong peptonization without coagulation	no peptonization or coagulation slight alkaline reaction	rapid coagulation and peptonization	peptonization without coagulation

	Streptomyces[1] *roseoflavus*	*Streptomyces*[1] *microflavus*	*Streptomyces* *ME/83*	*Streptomyces*[1] *parvus*	*Streptomyces*[1] *aurantiacus*
Nutrient agar	veg. golden yellow, aer. pinkish white, pig. absent	veg. colorless with yellowish reverse, aer. white, pig. yellowish	veg. melon to orange, aer. pinkish pig. absent	N.D.	veg. orange yellow, aer. absent pig. absent
Synthetic agar (Czapek-Dox)	veg. colorless to yellowish, aer. yellowish pink, powdery pig. absent	veg. colorless aer. white, trace, pig. absent	veg. pale melon, aer. pinkish white, trace, pig. absent	N.D.	veg. orange, aer. absent, pig. absent
Glucose — asparagine agar	veg. colorless to yellowish, aer. pink pig. absent	veg. yellowish aer. white, pig. absent	veg. deep orange pink, aer. pinkish white, trace, pig. yellowish	N.D.	veg. raspberry red aer. absent pig. absent
Glycerol — asparagine agar	N.D.	veg. colorless, pink reverse with green tinge, aer. velvety, white, pig. pinkish	veg. deep orange pink, aer. absent, pig. yellowish	N.D.	veg. orange with green iridescence aer. absent pig. absent

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[1] Data from (12).

[2] Waksman strain obtained from C.B.S.,; this strain is probably different from *S. microflavus* mentioned in (7).

[3] Data from (7).

[4] Description of local isolate.

[5] No data available.

Sporulation

S. mediterranei produces very few spores when grown on the media used for classification purposes. Any developing studies of a new streptomycete such as work with mutagens for the isolation of higher yielding strains, requires the preparation of fairly abundant spore suspensions. Efforts were therefore directed towards the finding of a solid medium more suitable for spore production. None of the proteic media tried would promote sporulation. Better results were obtained when mineral base media supplemented with various carbon sources were used. Sporulation took place on media containing 1 % glucose, lactose, glycerol and inositol (11). Separate sterilization of carbohydrates before addition to media did not prove advantageous. Best results were obtained on mineral base-lactose agar (ML) where the aerial mycelium assumed a rather mealy texture.

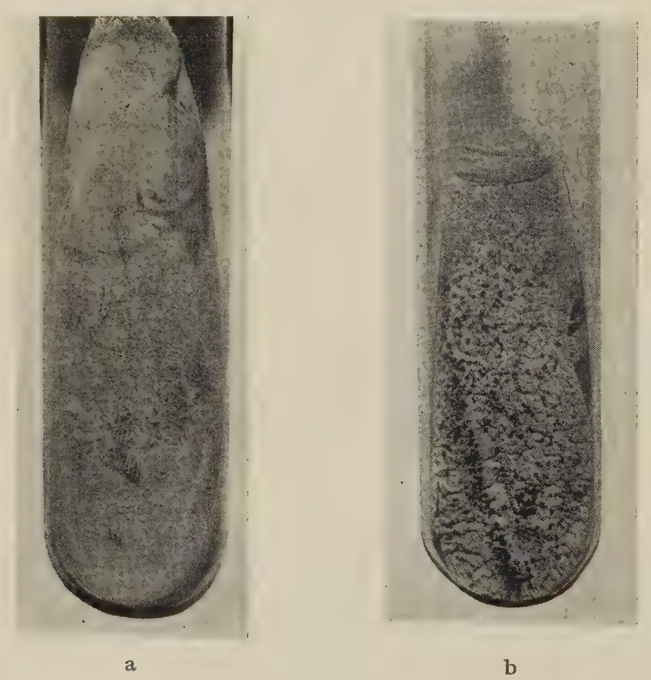
a b

Fig. 2. a) Normal culture; b. Degenerate culture with actinophage like spots.

The following procedure was used: *S. mediterranei* was grown for 48 hrs. in shake flask (500 ml with 100 ml seed medium) at 28° C. The mycelium was then separated by centrifugation, resuspended in the original volume of sterile water and a 10 ml aliquot was inoculated into the surface layer of a 250 ml. ML agar in Roux flasks. After the setting of the mycelium the excess of liquid was removed aseptically. Cultures were incubated at 28° C for 5—7 days. On washing the surface with 20 ml of a tween solution (1 : 10^4) and filtering through a glass filter to remove mycelial and spore conglomerates, a suspension of about 2×10^7 spores per ml was obtained. The ability of *S. mediterranei* to sporulate is one of the few macroscopic characters which are considerably variable due to mutagenic treatments. Thus we came across a strain ME/83/4008 which is a heavy sporulator, yielding under similar conditions up to 2×10^9 spores per ml.

Degeneration

S. mediterranei easily looses some of its characteristic morphological features when transplanted continuously on protein rich media. The aerial mycelium rapidly ceases to develop. Frequently the surface of a slant reveals transparent spots surrounded by whitish aerial mycelium reminding actinophage activity (see Fig. 2). When subculturing mycelium that lost the capacity to produce aerial hyphae, fresh cultures do not develop normally, the bare vegetative mycelium showing a strong tendency to lyse. Complete lysis was obtained when inoculating these cultures into shake flasks containing seed medium. In order to prevent the run down of this streptomycete, we used, exclusively, mineral base lactose sporulating medium on which the typical appearance of the organism could be maintained without difficulty.

SUMMARY

A new, antibiotic producing, *Streptomyces* has been isolated from a soil sample. A detailed description of its morphological and some of its biochemical characters are given. The name *S. mediterranei* nov. sp. is suggested for the new isolate. Sporulation of the new *Streptomyces* could be induced by cultivation on certain chemically defined media.

Bibliography

1. Sensi, P., P. Margalith, & M. T. Timbal — Farmaco (Ed. Sci.) **14, 146** (59).
2. Sensi, P., A. M. Greco & R. Ballotta — Antibiotics Annual 1959—1960 New York, Medical Encyclopedia, Inc. 1960 — p. 262.
3. Timbal, M. T. — Antibiotics Annual 1959—1960, New York, Medical Encyclopedia, Inc. 1960 — p. 271.
4. Maffii, G. & M. T. Timbal — Antibiotics Annual 1959—1960, New York, Medical Encyclopedia, Inc. 1960 — p. 277.
5. Furesz, S. & R. Scotti — Antibiotics Annual 1959—1960, New York, Medical Encyclopedia, Inc. 1960 — p. 285.
6. Furesz, S. — Antibiotic Medicine and Clinical Therapy. (in press).
7. Waksman, S. A. & H. A. Lechevalier — Actinomycetes and their antibiotics. Williams and Wilkins Co., Baltimore, 1953.
8. Waksman, S. A. — The species concept among the actinomycetes with special reference to the genus streptomyces. Bact. Reviews **21,** 1 (1957).

9. Maerz, A. & M. R. Paul — A dictionary of color — 2nd edition McGraw-Hill Book Co. 1953.
10. Hickey, R. J. & H. D. Tresner — A cobalt containing medium for sporulation of streptomyces species. J. Bact. **64,** 891 (1952).
11. Pridham, T. G. & D. Gottlieb — The utilization of carbon compounds by some actinomycetales as an aid for species determination. J. Bact. **56,** 107 (1948).
12. Tadashi Arai — Studies of Flavomycin. J. Antib. **4,** 215 (1951).

A NEW SPECIES OF CATENARIA PARASITIC ON NEMATODES OF SUGARCANE

by

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(11.V.1960)

A review of the literature showed that records of the fungus *Catenaria anguillulae*, a parasite of nematodes, date back at least to the work of Braun (1) in 1856 and probably beyond to a fungus described under the name of *Chytridium zootocum*. In 1874 Villot (20) published on an organism described as a fresh-water alga which might have been closely related to the presently described *C. anquillulae*. Sorokine (17) is credited with the name *C. anguillulae* for his description and figures in 1876. Dangeard (8, 9) rediscovered what he considered to be this same fungus in 1885-1886, but the zoospore measurements were larger than those by Sorokine. In 1901 Constantineanu (6) identified *C. anguillulae* from diseased nematodes in Rumania but with different-sized sporangia and sporangial tubes. In 1907 Serbinov (16) described a new species named *Catenaria pygmaea*, but its validity was questioned by later workers (3,7). In 1928 Butler (3), in a review of the literature on *Catenaria species*, published detailed morphology of *C. anguillulae* as it occurred in liver-fluke eggs. In 1932 Butler & Humphries (5) first cultured the fungus on an artificial medium. In the same year Sparrow (18) reported *C. anguillulae* as a probable parasite of rotifers at Cold Spring Harbor, Michigan. Karling (13,14) made a further study of *Catenaria* and described *C. spherocarpa* as a saprophyte of plant tissues, differing from *C. anguillulae* primarily in the morphology of the zoospores and the sporangia. He was unable to parasitize root-knot nematodes of tomato (apparently *Meloidogyne* sp.) with *C. spherocarpa*. In 1945 Couch (7) described a new species, *Catenaria allomycis*, from *Allomyces anomalus*. He stated that the thallus of this fungus was similar to that of *C. anguillulae*. For comparison Couch isolated *C. anguillulae* from fluke eggs in Texas soil and grew it on a variety of media, as well as on nematodes, pointing out the great variation in zoospore and sporangial measurements reported in the literature.

In reviewing the literature one is indeed impressed by the differences in size, shape, and structure of the zoospores and sporangia of what presently includes *C. anguillulae*. Probably 3 or 4 different species are lumped under this name. Certainly it seems justifiable to question the abnormal variation in one species unless the substrate could be responsible. It may be that if all the descriptions of *C. anguillulae* were based on nematodes as a source of food, zoospore and sporangial measurements would be more consistent.

The taxonomic position of the genus *Catenaria* has varied considerably. SOROKINE (17) suggested a relationship to *Achylogeton* SCHENK. in the *Chytridiales*, while DANGEARD (9) placed it in the *Ancylistales*. Other workers including FITZPATRICK (12), KARLING (14), and SPARROW (19) placed the genus in the *Chytridiales*. In 1945 COUCH (7) erected a new family *Catenariaceae* from the subfamily *Catenarioidae* SPARROW and removed it into the order *Blastocladiales*.

The purpose of this paper is to show the mode of infection, parasitism, pathogenicity, and kinds of plant-parasitic nematodes destroyed by a new species of *Catenaria* of apparent economic importance in the sugarcane soils of Louisiana. BUCKLEY & CLAPHAM (2) recognized the apparent economic importance of *C. anguillulae* to helminth eggs as early as 1929, but its economic relationship to plant-parasitic nematodes has been entirely neglected.

The new species of *Catenaria* described here differs from *C. anguillulae* primarily in the absence of rhizoids, (observed on artificial media), smaller size of zoospores, and the more extensive septate mycelium between the sporangia. Since only thick-walled sporangia were observed this would suggest a new type of ife cycle in the Catenariaceae.

Catenaria vermicola sp. nov.

Thallis endobioticis, polycentricis, in nematoideis pluribus plantas parasitantibus et saprogenis, e myceliis septatis sine rhizoideis in catenas sporangiorum tumentibus compositis; septis completis foveolatis, spatio inter septa circa 5.8—45.1 μ; myceliis sporangia pauca vel numerosa, spissa, bitunicata, 16 × 18 usque 24.4 × 40.2 μ, tubis sporangialibus apta efformantibus; zoosporis e sporangiis germinantibus natis, ovoideis, 1.3 × 2.6 usque 3.1 × 4.2 μ, mediis inter maximum minimumque circa 2.6 × 3.1 μ, granulas 3—4 praeditis, posteriore uniflagellatis aut sine flagellis et motum amoeboideum exhibentibus. Flagellis in longitudine diametrum zoosporarum bis aut tris multiplicantibus, quandoque multo maioribus per tubam interiorem sporangialem singulis aut in massa gelatina emergentibus; tubis sporangialibus cuticulam dissolventibus vel zoosporis endobioticis emittientibus. Folliculis zoosporarum globosis, circa 2 × 3 usque 3 × 3 μ, parietibus circa 2 μ, spissis, protinus germinantibus et quod intus continetur in cuticulam nematoideorum deponantibus. Type host: *Xiphinema chambersi* THORNE, 1939. Type locality: Shreveport, Louisiana. Type specimen No. 71634 in U. S. Department of Agriculture National Fungus Collections. Paratypes in the New York Botanical Garden and at Royal Botanic Garden, England.

Catenaria vermicola

Thallus endobiotic, polycentric, parasitic to several plant-parasitic and saprophagus nematodes, consisting of a septate mycelium without rhizoids, (except on artificial media), which swells to form chains of sporangia. Distance between septa about 5.8—45.1 μ, septa completely formed, pitted, mycelium forming few to abundant, double-walled sporangia, 16 × 18 to 24.4 × 40.2 μ, smooth with sporangial tubes. Sporangia germinating and forming zoospores, which are ovate 1.3 × 2.6 to 3.1 × 4.2 μ. Average about 2.6 × 3.1, with 3 or 4 granules, posteriorly uniflagellate or without flagella in which case movement is amoeboid. Flagella 2 or 3 times the diameter of zoospore in length but they may be much longer, emerging through the inner sporangial tube in a gelatinous mass. Sporangial tubes dissolve cuticle or may discharge zoospores endobiotically. Cysts of zoospores spherical, about 2 × 2 to 3 × 3 μ, wall relatively thick, germinating directly and depositing contents inside cuticle of nematodes.

STUDIES ON THE PATHOGENICITY OF CATENARIA VERMICOLA ON PLANT-PARASITIC NEMATODES OF SUGARCANE

Catenaria vermicola was first collected by Dr. JOHN HOLLIS on *Xiphinema chambersi* from St. Augustine grass in the Shreveport area of Louisiana. Subsequent studies have shown its abundance in sugarcane soils and in at least one undetermined location in the rice area of Louisiana. Although ring nematodes of the family *Criconematidae* appeared to be more resistant than several other species observed, the fungus showed very little specificity, the most important factor apparently being the physical vigor of the exposed animals. Healthy specimens of *Tylenchorhynchus martini* subjected to the fungus in syracuse watchglasses became visibly infected in 3—4 days. In about 7—10 days the epidemic spread to most of the exposed population.

Diseased specimens among nematodes recently extracted from the soil were removed, with the aid of a stereoscopic microscope and dental pulp needle, to a hanging drop for study and observation. Some of the cover slips were sealed with a ringing compound called "zut". Some of the diseased material was killed in formalin-alcohol acetic acid and stained in lactophenol acid fuchin.

Material for study was maintained in a syracuse watchglass by feeding the fungus live specimens of nematodes.

A beef-liver extract as employed by COUCH (7) for the culture of *Catenaria anguillulae* was used extensively to grow *C. vermicola.* This medium differed from one employed by COUCH only in that it was not strained through blood charcoal. The formula used with considerable success was as follows: one lb. of beef liver was ground and the juice extracted, strained, boiled 10 minutes, and strained a second time. 140 ml of the extract, with 20 g of sucrose and 15 g of agar, were used to make one liter.

Nematodes are primarily infected by zoospores which swim and encyst near the oral opening, vagina, and the anus of the animals where the cuticle is apparently structurally weak (Fig. 2, I). In a few hours, if not dislodged by the movement of the victims, a germ tube penetrates the cuticle and grows into the body leaving the empty cyst intact. Inside the body the germ tube enlarges,

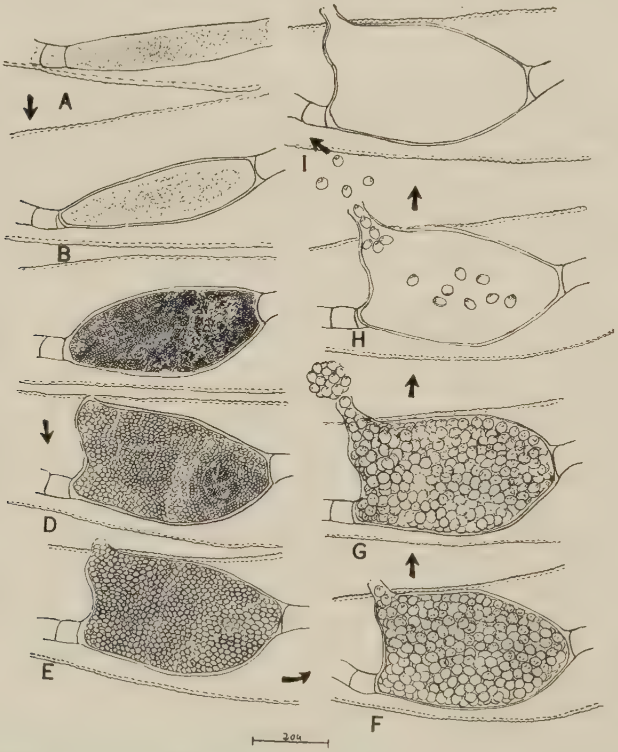

Fig. 1. Stages in sporangial and zoospore development of *Caternaria vermicola* in a plant-parasitic nematode: A) Early stage of sporangial formation, B) Swelling of the sporangium and forming of septa, C—F) Zoospores forming in the sporangia, D—F) Sporangia neck emerging through the nematode cuticle, G) Mature zoospores emerging through sporangial neck and forming a cluster, H) Escaping zoospores, I) Empty sporangium.

apparently strongly absorbing food, and forms prominent septa that appear to be of perforate nature (Fig. 2, A). Areas between some of the septa begin to enlarge as a result of the flow of mycelial contents from adjacent cells, and the protoplasm becomes dense, swells and forms double-walled sporangia (Fig. 1, A—C). The sporangia are spindle-shaped at first, but as they grow they assume various shapes from spherical to subspherical, flask shaped, ovate, oblong, clavate, obclavate to irregular. After 2—3 days cleavage occurs and the outline of zoospores can be seen in some (Fig. 1, D—H), while in others the contents draw away from the walls, ball up and remain as a resting spore, finally germinating to form zoospores as usual (Fig. 2, B). A few hours after zoospores are observed in the sporangia a place in the primary wall balloons out, usually toward the cuticle. When contact is made the cuticle

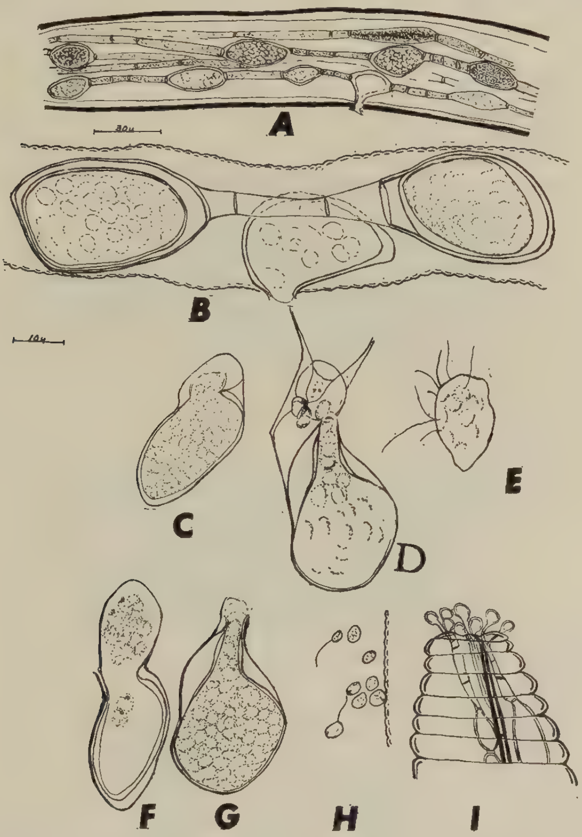

Fig. 2. *Catenaria vermicola*-infection of plant-parasitic nematodes. A—B) Hyphae and sporangial development in *Xiphinema chambersi*. C—G) Sporangial and zoospore development. H—I) Infection of *Criconemoides* sp.

dissolves and the primary wall breaks at this point (Fig. 1, D—G). The secondary sporangial wall forms a papilla, grows into the provided opening to the surface, and discharges the zoospores in the gelatinous mass, gradually extracting themselves. Except for a few which are generally left trapped inside the sporangia, they then swim quickly away (Fig. 3, B). Zoospores are equipped with long posterior flagella which are difficult to observe. The zoospores lose or retract their flagella, (I have not observed which of these phenomena occurs). Then they lodge on the nematode cuticle. They

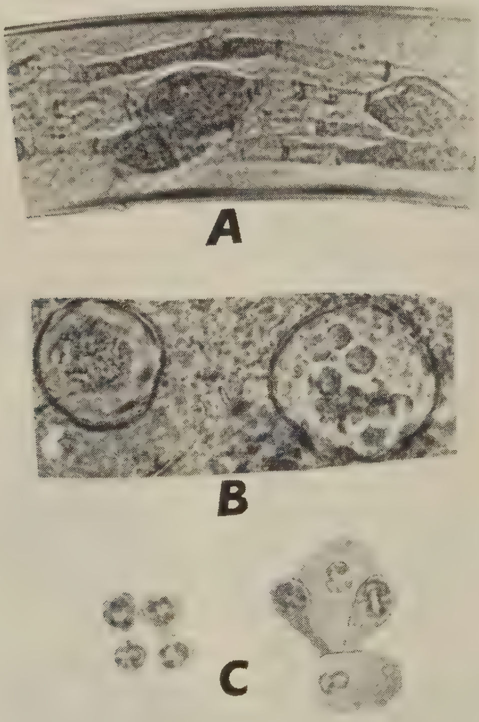

Fig. 3. Photomicrograph of *Catenaria vermicola*.
A—B) Hyphae and sporangia in *Xiphinema chambersi*.
C) Zoospores with and without visible flagella.

encyst and germinate by a germ tube and reinfect a dead nematode or infect living specimens. Those trapped inside the sporangia encyst, but I have not observed their germination. Sometimes the sporangial contents exude in mass without complete cleavage and form zoospores (Fig. 2, D, E). These masses, composed of many nuclei in clear plasmodia, break up into smaller portions (5.5—8μ) with 2 or more nuclei. They move about slowly by several pseudopodia, which resemble flagella, reminescent of small amoebae (Fig. 2, D). In about 12—23 hours these plasmodial masses round up and form cysts as do the zoospores; germination was not observed although several were seen to lodge on the cuticle of diseased nematodes. It is possible that plasmogamy and karyogamy take place in this manner but these were not observed inside the parasitized nematodes. Sporangia continue to form from secondary infection so that finally nothing remains of the victim except cuticle and cuticularized structures such as the stomatostyle. The sclerotized cephalic regions of *Pratylenchus zeae* and *Hoplolaimus tylenchiformis* were not utilized by the fungus.

The following plant-parasitic nematodes were attacked and destroyed by *Catenaria vermicola*: *Belonolaimus longicaudatus*, *Criconemoides rusticum*, *Hemicycliophora gigas*, *Hoplolaimus tylenchiformis*, *Meloidogyne* larvae, *Pratylenchus zeae*, *P. brachyurus*, *Radopholus similis*, *Tylenchulus semipenetrans* larvae, *Tylenchorhynchus martini*, and *Xiphinema chambersi*.

The economic importance of this fungus appears to be considerable; therefore further studies are in progress.

SUMMARY

A new species, *Catenaria vermicola*, is named and described. It attacks several plant-parasitic nematodes of economic importance in sugarcane soils of Louisiana. The fungus forms numerous zoospores which encyst on the cuticle, generally near natural openings of the nematode, thereby causing infection. Within the parasitized nematodes hyphae and sporangia quickly form and destroy the victim completely. The following plant-parasitic nematodes were attacked and destroyed: *Belonolaimus longicaudatus*, *Criconemoides rusticum*, *Hemicycliophora gigas*, *Hoplolaimus tylenchiformis*, *Meloidogyne* larvae, *Pratylenchus brachyurus*, *P. zeae*, *Radopholus similis*, *Tylenchulus semipenetrans* larvae, *Tylenchorhynchus martini*, and *Xiphinema chambersi*.

Literature Cited

1. BRAUN, A., 1856. Über neue Arten der Gattung *Chytridium* and über die damit verwandte Gattung Rhizidium. Mber. Akad. Wiss. Berlin. p. 591.
2. BUCKLEY, J. J. C. & P. A. CLAPHAM, 1929. The invasion of helminth eggs by chytridiacean fungi. J. Helminth. **7**: 1—14.
3. BUTLER, E. J., 1928. Morphology of the Chytridiacean fungus *Catenaria anguillulae* in liver-fluke eggs. Ann. Bot. **42**: 813—821.

4. Butler, J. B. & J. C. Buckley, 1927. *Catenaria anguillulae* as a parasite of the ova of *Fasciola hepatica*. Sci. Proc. R. Dublin Soc. **18:** 497—512.
5. Butler, E. J. & Annie Humphries, 1932. On the cultivation in artificial media of *Catenaria anguillulae*, a chytridiacean parasite of the ova of the liver-fluke, *Fasciola hepatica*. Sci. Proc. R. Dublin Soc. **20:** 301—324.
6. Constantineau, J. C., 1901. Contributions à la flore Mycologique de la Roumanie. Rev. Gén. Bot. **13:** 369—389.
7. Couch, John N., 1945. Observations on the genus *Catenaria*. Mycologia **37:** 163—193.
8. Dangeard, P. A., 1885. Note sur le *Catenaria anguillulae* Sorok. et *Chytridium zootocum* A. Br. Bull. Soc. Linn. Normandie, **9:** 126—135.
9. Dangeard, P. A., 1886. Recherches sur les organismes inférieurs. Ann. Sci. Nat. **4:** 241—341.
10. Dangeard, P. A., 1911. Un nouveau genre de Chytridiacees. Bull. Soc. myc. France **27:** 200—203.
11. Fischer, A., 1892. Die Pilze Deutschlands, Oesterreichs, und der Schweiz. in Rabenhorst's Krypt. Fl. 1, Abt. 4. Leipzig. P. 143—144.
12. Fitzpatrick, Harry Morton, 1930. The lower fungi Phycomycetes. McGraw-Hill Co., Inc., New York. 331 pp.
13. Karling, John S., 1934. A saprophytic species of *Catenaria* isolated from roots of *Panicum varigatum*. Mycologia **26:** 528—543.
14. Karling, John S., 1938. A further study of *Catenaria*. Amer. J. Bot. **25:** 328—335.
15. Minden, M., 1911. Chytridineae. Krypt. Flora. Mark Brandenburg **5:** 418—419.
16. Serbinop, J. L., 1907. Organisation and Entwicklungsgeschichte einiger Chytridineen-Pilze (Chytridineae, Schröter). Scripta Bot. Hort. Universitatis Imperiales Petropolitanae, fasc. **24:** 84—93.
17. Sorokine, N., 1876. Note sur les végétaux parasites des anguillulae. Ann. Sci. Nat. **4:** 62—71.
18. Sparrow, F. K., 1932. Observations on the aquatic fungi of Cold Spring Harbor. Mycologia **24:** 268—301.
19. Sparrow, F. K., 1943. Aquatic Phycomycetes. Ann Arbor, Michigan, p. 320.
20. Villot, A., 1874. Monographie des Dragonneaux. Arch. Zool. expér. gén. **3:** 184—185.
21. Whiffen, Alma J., 1944. A discussion of taxonomic criteria in the Chytridiales. Farlowia **1:** 583—597.

MEDICAL MYCOLOGY IN INDIA

BY

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(12.IV.1960)

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The survey of the literature pertaining to this review was completed in September 1959.

INTRODUCTION

In 1876 when the epoch making discoveries of KOCH and PASTEUR proved that bacteria (*Bacillus anthracis*) can be specific etiological agent of Anthrax and the germ theory of disease was firmly established, many fungi had already been recognised as plant and animal pathogens. The development of highly improved compound microscope (ABBE 1860—1890) synchronising with the series of brilliant bacteriological discoveries held the attention of scientists so much that the role of fungi in human diseases was relegated to the background. The reverse was the case with plant diseases. Since BERKELEY (1846) and DE BARY (1853) proved the parasitism of fungi associated with potato blight, and rusts and smuts of wheat, respectively, fungi, in turn, received the most attention and continued to do so even after the demonstration of bacterial origin of fire blight of pears by BURRILL (1878—1881). The reasons are not far to seek. The great majority of the more important human diseases are of bacterial origin and those of plants are due to fungi.

As far as we know Agostino BASSI in 1835 put forth the first experimental proof that the muscardine disease of silkworm was caused by a fungus now known as *Beauvaria bassiana* (AINSWORTH 1951). Similar etiological relationship between fungus and man was established when in 1839 mycotic origin of favus was demonstrated by SCHOENLEIN and that of thrush by LAGENBECK. It was, however, the monumental work of SABOURAUD (1864—1938), who may be aptly called the father of medical mycology that placed this discipline on the map of world science. Since then a large number of distinguished workers world over have made a significant contribution on various aspects of the subject.

Medical research in India owes its beginning to the pioneering efforts of a selected band of British medical officers in service in India who initiated investigations on diseases prevailing in the country. So far as mycotic diseases are concerned it was H.V. CARTER who in 1860 first established that a disease characterised by the peculiar swelling of the foot, named by him Mycetoma in 1861, was of mycotic origin. There followed further contributions by RUSTOMJI (1860), EYRE (1860), H.V. CARTER (1874a), D. F. DYMOCK (1881), J. MAITLAND (1898), all on mycetoma. D. D. CUNNINGHAM (1873) carried out experiments to determine whether correlation could be established between the daily spore content of the air and the incidence of five diseases (ague, dysentry, diarrhoea, dengue and cholera) in the Presidency Jail, Calcutta. This study, among the first aerobiological surveys of fungus spore, coupled with the classical work of C. H. BLACKLEY (1873) on hay fever in England led to the recognition of fungus spores as one of the airborne allergens associated with asthma and similar other allergic conditions. It was not until 1900 that a second mycotic disease, i.e. Ringworm was described by POWELL from Assam. CASTELLANI in 1905 reported Dhobies itch, *Epidermophyton* being the etiological agent.

The first world war brought into prominence and stimulated research in mycotic diseases in India leading to the establishment of School of Tropical Medicine in Calcutta in 1927. Recently it has gained added impetus due to the appreciation of the fact that fungi play a more significant role in human disease than so far credited. Several centres are now actively engaged in research in various aspects of medical mycology. The more important among these being School of Tropical Medicine, Calcutta; Government Stanley Hospital, Madras; K. E. M. Hospital, Bombay; Mycology School, Botany Department, Lucknow University; Central Drug Research Institute, Lucknow; S. M. S. Medical College, Jaipur; and Ballabhbhai Patel Chest Research Centre, New Delhi. Besides these, isolated work is being carried out in several medical institutions. The recent (1959) symposium on 'fungus diseases in India' organized by the School of Tropical Medicine, reflects the growing interest of both medical men and mycologists in the subject.

Several reviews and general articles have appeared in India on mycotic diseases such as 'Rhinosporidiosis' by ALLEN & DAVE (1936); 'Chromoblastomycosis' by ANDLEIGH (1953); Pulmonary diseases by the same author (1951, 1958) : Study of Skin diseases Communicable to Man' by CHAKRABARTY, GHOSH and BANERJEE (1953), 'Ringworm of Hair' by DEY (1953), 'Granuloma in India' and a text book on medical mycology by the same author (DEY 1959, 1959a). RAO (1959) under the title 'Fungus Diseases in India' has contributed a good summary of the mycotic diseases as seen in the skin clinic of the School of Tropical Medicine, introduced by an editorial article on 'Fungus diseases in man' by R. N. CHAUDHURI.

The present review is an attempt to gather together all the available contributions on medical mycology in India from its inception right upto 1959. It deals with the human mycotic diseases both superficial and systemic (deep) and also with the nutritional, physiochemical and chemotherapeutic studies of the causal pathogens. In the absence of proper mycological approach the authenticity of some of the records may appear doubtful but nevertheless the works are valuable contributions to our knowledge of mycotic diseases in India.

MADUROMYCOSIS

(Madura foot or Mycetoma)

Maduromycosis is a chronic infection affecting usually the feet and rarely the hand or other parts of the body. It is caused by a variety of filamentous fungi of different species and genera and is characterised by the development of tumifactions and sinuses. The infection is present in all parts of the world and has been reported from various parts of America, Asia, Africa and Europe. In India it is endemic in South India and in desert part of Central Provinces and was first recorded in Madura district, Madras state, but since then it has been reported from different parts of the country.

Madurafoot is the first human mycotic disease to be recorded from India. Besides the reference by COLLAS (1861) that French missionaries in Pondicherry have mentioned a disease akin to madurafoot as far back as 1714, the earliest original work available to the authors from which historical data could be drawn is that of H. V. CARTER (1860) "On a new and striking form of fungus disease, principally affecting the foot and prevailing endemically in many parts of India". These data have been considered here in some detail as there exist certain discrepancies in the statements in some current literatures.

We learn from CARTER'S statement in 1860 that GILL of Madura clinic in his report for 1842 gave a short account of the disease describing a condition of foot characterised by marked deformity with offensive serous discharges (Madura Dispensary Report, 1842). This was referred to by COLEBROOK in his Madras Dispensary Report of 1848 where he succeeded GILL as 'Jillah' (District) Surgeon, Madura. In this report COLEBROOK drew attention to the term 'Madurafoot' derived from the locality where it was first observed. In the meanwhile J. GODFREY, Garrison Surgeon at Bellary, Madras Presidency in 1844 furnished a detailed account of the disease from Bellary district and further described four cases in 1846 which included two cases encountered in 1845. He suggested the name '*morbus tuberculosus pedis*' for the disease. In succeeding years there were several reports of the disease dealing mainly with the clinical features. Of these BRINGAL in 1859 in his paper entitled "An account of the tumor affecting the foot" gives the first notice of the microscopic peculiarities of the disease. He described that ,,pulpy granular substance found in the internal part and the discharge when microscopically examined were found to be composed of large granular cells, circular or oval in nature and generally surrounded with transparent fringes consisting of large irregular spiculae. Radiating groups of spiculae without any distinct cell with numerous oil globules in the centre were also detected". The nature of these bodies could not be explained by the author, which apparently was responsible for the disease. RUSTOMJI (1860) differentiated two forms of the disease, one melanoid showing a dark thick deposit the other ochroid showing soft yellowish granules. EYRE (1860) described the external symptoms of the disease, its history, natural course, morbid anatomy, etiology and habitat. COLLAS (1861) described black maduromycosis as seen in Pondicherry. He recognised the little bodies of blackish or reddish brown colour appearing in clearer part as small transparent cells which he could not properly study.

H. V. CARTER (1860) while he was the Professor of Anatomy and Physiology at the Grant Medical College, Bombay, had made a special study of this disease. On the basis of the structure and certain other distinguishing features, he considered "it correct to look upon the organism as 'fungi', although their habitat appeared to be very unusual one for fungi" and thus "established the pathology of the disease leaving no doubt as to these fungi being the existing cause of the disease". CARTER also recognised the existence of more than one strain of the fungus. Describing in detail the fungi obtained from different sources he distinguished two varieties (pale and black) and concluded that "although fungus responsible for the two varieties of diseases differ materially, yet there are indications of transitional forms and the conclusion that the two varieties are closely related will not appear to be far fetched."

In (1861) CARTER described new cases from Bombay (Jamsetji Hospital), Bengal Presidency, North Eastern shores of Persian Gulf, Kutch, Kurachee and suggested its inclusion in the nosological list of Indian diseases as a specific malady. The same year (1861)

CARTER suggested the term 'mycetoma' for the disease "for the sake of convenience and distinction when referring to their peculiar form of swelling". The author, however, considered it premature to naming the fungus of either variety of mycetoma. Specimens of this disease were sent to England for expert examination by Rev. M. J. BERKELEY who found a phycomycetous mould associated with the black granules which he named as *Chionyphe Carteri* (BERKELEY, 1863, 1863a) which CARTER himself in 1886 considered to be a contaminant and drew attention to the similarity between the ray fungus discovered by HARZ in 1877 and that of mycetoma. In 1874 CARTER wrote a monograph on mycetoma or the fungus disease in India giving a comprehensive account of the disease followed by another contribution (CARTER 1874a) on the nature of the disease.

The next few records of human mycotic diseases are also on madurafoot. HOLMSTED (1870) in Hyderabad, Sind, found a thorn in a case of black mycetoma which was embedded for two years in the affected area. The same year BRISTOWE (1870) described and illustrated a fungus seen in the black particles of foot affected with black maduromycosis und amputated in Cantoor. His description confirms CARTER'S work. HOGG (1872) described a black maduromycosis in India in which he was able to observe fungal threads. He, however, believed that the fungus was a secondary product and did not originate the disease. DYMOCK in 1881 recorded a case revealed to be madurafoot by microscopic examination of diseased parts. It was a coolie at Vellore whose foot amputated due to abnormal swelling had duly healed but later on developed tumours on the stump and on the thigh. BOCCARO (1893) reported his analysis of one hundred cases of mycetoma of which 8 were females and the rest males; the disease prevailing mainly among muslims there being a definite occupational relationship. He further discussed the diagnosis, prognosis of the disease with the remark that local infecting agents, such as, injury, prick of thorn, appeared to be a common history of the cases examined. This was followed by another report (BOCCARO 1894) showing the common occurrence of the disease in Sind affecting generally muslim cultivators or people exposed to such conditions. KANTHACK (1893) studied yellow and black mycetoma with the comment that the yellow variety agreed with actinomycosis and the black granules were the same organisms only in a degenerated condition. BOYCE & SURVEYOR (1894) finally established that the organisms of the black and yellow varieties are dissimilar and created two divisions of the disease actinomycosis and maduromycosis which have since been accepted. VINCENT (1894) as reported by AINSWORTH (1951a) recognised the aerobic Actinomycetes *Streptothrix Madurae* now *Nocardia Madurae* VINCENT, as the cause of the classical madurafoot. J. SMITH (1898) recorded mycetoma of the neck of a young hindu merchant of Trichinopoly. Neck showed swelling and was studded with blebs which internally led by tortuous sinuses towards the spinal column. On puncture, the blebs oozed out pus and rod like bodies which the author failed to cultivate. MAITLAND in the same year (1898) described a mycetoma of abdominal wall of a hindu male of 45 years. The chief point of interest being its unusual situation which the author regarded as an extension from the original focus amputated 11 years before.

K. PRASAD (1906) described madurafoot of a middle aged Burmese farmer. His right foot was swollen, riddled with sinuses and emitted foul smell. A previous history of wood splinter injury ten or twelve year ago appeared to be the cause of the trouble. Microscopic findings were the basis of diagnosis. F. J. PALMER described two instances of madurafoot, confirmed microscopically and culturally. The first was of an Indian soldier from the North West frontier province (PALMER 1926) and the other of an elderly Indian soldier affecting the right ankle region (PALMER, 1928). He discussed the chemotherapuetic treatment as an apparent means of curing the disease. BRADFIELD & VASUDEVAN (1927) reported the successful treatment of mycetoma of the right foot by excision of the great toe and metatarsal bone in a hindu farmer from Madras. In the process of recovery a small subcutaneous nodule of the size of a pea was found above the right thumb and another similar one below malleolus. The nodules were found to contain granules giving positive culture which proved to be black mycetoma. The disease was common over the whole of Madras district with no specific endemic area.

RAO (1928) reported a mycetoma of the hand and foot in a resident of Valavampatti with characteristic swelling of the parts affected showing multiple sinuses exuding pus. AHMAD (1929) recorded a madurafoot from a resident of Punjab, who showed a swelling of the right foot running up to the ankle joint riddled with numerous sinuses discharging profuse offensive pus containing typical mycetoma granules. Amputation proved to be the correct treatment with relief. VASUDEVAN & SESHADRINATHAN (1929) gave a brief account of the disease and carried experiments to find out the mode of infection. They successfully cultured the pathogenic fungus on tender bamboo twigs, succulent stem of common prickly pear and on soil. They think it possible that the fungus resides saprophytically on these thorns and soil and enter subsequently through the wounds. FITZERALD (1932) reported a mycetoma like condition of the foot. TRIBEDI & MUKHERJEE (1939) described three cases of maduromycosis from Calcutta Medical College, of which two were due to *Actinomyces Madurae* with ochroid type of granules, while the third was complicated by a *Staphylococcus aureus* infection. The pathogen isolated from these patients was cultured, described, and identified (identification of the fungus of the third patient is not mentioned). RAY & TRIBEDI (1938—39) published a case of mycetoma of the breast with a brief discussion as to the source and mode of infection of the disease. GHOSH, DEY & PANJA (1950) described in detail two more cases of madurafoot and according to them this disease is commonly prevalent in farmers or in people who do field work bare footed. Mycetoma caused by the fungus *Glenospora Semoni* has been described by KAKOTI & DEY (1956) along with the morphological and cultural character of the pathogen and the diagnostic characters of the disease.

ANDLEIGH (1957) has described the etiology of maduromycoses in India and has critically discussed the thirty isolates of different fungi as its etiological agents together with their cultural habit, animal pathogenicity and classification. Besides this ANDLEIGH (1957a) has also carried out *in vitro* tests of the antifungal activity of Pentamidine and Stilbamidine. He found that Pentamidine in concentration of 0.1 mg/ml. of the medium inhibited the growth of *Madurella mycetomi* (causing maduromycosis). Stilbamidine does not hold any promise as an effective chemotherapeutic agent. PANJA & CHAUDHURI (1958) recorded a black mycetoma caused by *Glenospora Semoni* from Bengal where the disease is rare. A detailed radiological account of various mycetoma was given by BASU, BANERJEE & RAO (1959) to explain certain clinical peculiarities of the disease.

Superficial mycoses

(Dermatomycoses and allied diseases)

Dermatomycoses are fungus infections of the superficial skin and its appendages caused by a well defined group of fungi, the dermatophytes comprising of various species of three genera, viz., *Trichophyton*, *Microsporon* and *Epidermophyton*. The investigations in dermatomycoses, which is by far the commonest of all the mycotic diseases in India, has received greatest attention, progressing along several lines and therefore, for the sake of convenience the contributions have been considered under three sub-heads. (A) Survey — dealing with clinical aspects, incidence, epidemiology and etiology of the disease; (B) Therapeutics — dealing with the studies of the action of drugs, chemicals (chemotherapy) and antibiotics on the pathogens responsible for superficial mycoses *in-vivo* and *in-vitro*; (C) Physiology — dealing with the nutritional, enzymic and other functional aspects of the causal pathogens.

A. Survey

The credit of the first report on dermatomycoses from India goes to POWELL (1900) who showed the prevalence of ringworm diseases in Assam with the complete absence of scalp ringworm in marked contrast to the then published records from Britain. He suggested that the anatomical distribution of the diseases (*Tinea*) is mainly influenced by the forms of dress worn by the patients in Assam and discussed the rare occurrence of ringworm before puberty while it was common in infants and children in Europe. The pathogenic fungi was classed as *Megalosporon* according to SABOURAUD'S classification. He noted the absence of *Tinea imbricata* in that state. POWELL (1900) also recorded, *Tinea versicolor* of cheek and chin for the first time in India contrary to the view of its non-incidence in face as reported by contemporary dermatologists.

CASTELLANI (1905) reported for the first time *Epidermophyton* as the etiological agent for *Dhobies itch* and further reported *Tinea imbricata* from Ceylon in 1914 and other cases in 1915 due to *Endodermophyton concentricum* and *Endodermophyton indicum* (CASTELLANI 1920). These fungi were successfully cultivated and their pathogenicity proved by inoculating their pure culture as well as the infected scales on human beings producing the typical disease. CASTELLANI also reported *Endodermophyton castellani* causing *Tinea intersecta.*

DEMELLO (1917—18) isolated a new species of *Trichophyton vianne* from the body of a Goanese soldier the first of its kind to be isolated from any Goanese suffering from mycoses. He also described the cultural characteristics of the fungus. G. PANJA (1925) described a case of melanoderma due to *Malassezia* spores with the results of the treatment undertaken.

H. W. ACTON in 1919 in an investigation of throat infections occurring amongst British and Indian troops of the Mesopotamian expeditionary force found the great prevalence of vincents and mycotic organisms. ACTON subsequently reported in another paper the same year (1919a) that mycotic infections of the throat commonly occur in the tropics and are due to *Endomyces tropicalis*. He reproduced the disease successfully by smearing the culture on the back of the throat and reported it to be the cause of pharyngitis and fatal type of bronchopneumonia.

ACTON & MCGUIRE (1927) reported the etiological agents *Epidermophyton floccosum* and *Epidermophyton rubrum* from *Tinea cruris* infections. According to them their manifestions are not recognised by medical men in India when other parts of the body are attacked and suggested that the means of identification is by microscopic examination and successful cultivation. ACTON & MCGUIRE (1929) also isolated *Trichophyton violaceum* var. *indicum* from the lesion of hair follicles of poor class of men and women in India who commonly suffer from this disease which is named as *Coolie itch* of *purulent folliculitis.*

ACTON & G. PANJA (1927) described three distinct types of lesions caused by three distinct species of *Malassezia* and stated that PANJA had successfully cultured this fungus in Petroff's glycerinated medium after many failures in other mediums.

ACTON & GHOSH (1934) reported for the first time *Tinea imbricata* occurring in a Bengali youth aged twenty years from Mymensingh. Successful culture of *Tinea imbricata* on both solid and liquid mediums was found possible only on planting fresh scales.

The authors suggested *Achorion concentricum* BLANCHARD (BLANCHARD 1896) as the correct nomenclature of the fungus *Tinea imbricata* and regarded the differentiation of the genus by creation of the genus *Endodermophyton* by CASTELLANI to characterise this genus as unjustified. The fungus causing this disease is at present known as *Trichophyton concentricum.*

ACTON & DEY (1934) described a method of recovering typical cultures of dermatophytes from pleomorphic forms by using feathers chemically and anatomically comparable to human hair and skin.

DEY & MAPLESTONE (1935) examined 53 cases of ringworm of the scalp. Of these two were due to *favus*, 20 were due to *Trichophyton violaceum* and the rest were caused by a commonly occurring fungus *Microsporon Audouinii*. This is the first detailed report of the occurrence of *Trichophyton violaceum* in India. Authors have suggested that the organism would be more correctly classed as *Achorion* given to it by BODIN in 1902. DEY & MAPLESTONE (1936) cultured successfully the pathogen by obtaining material from a boy living in Rajputana. The fungus is similar to *Achorion Schoenleinii* but the name *Achorion Actoni* n. sp. has been suggested because of its possession of additional funnel shaped chlamydospores. The present taxonomical relationship between certain species of *Trichophyton* and those of *Achorion* still remains to be clarified.

MAPLESTONE & DEY (1939) regarded microsporosis as rare in the plains of India. They reported successful isolation in culture of *Microsporon Audouinii* and of *Microsporon ferrugineum* from hairs of four boys and of six girls and one boy, respectively. *M. ferrugineum* appeared to be new and first report from India. GHOSH & MAPLESTONE (1940) described a rare case of body ringworm of a female hindu child of 7 months. External symptoms indicated *Epidermophyton* infection. This was confirmed by the isolation in culture of *Epidermophyton floccosum* from scales obtained from ring shaped patches on the right shoulder. In 1940 MITRA described Otomycosis in a woman of 48 years due to *Aspergillus niger* TIEGHEM.

DEY & MAPLESTONE (1941) reported for the first time in India the occurrence of *Tinea tonsurans*. They isolated *Trichophyton crateriforme* from the scalp of all Anglo-Indian girls aged between 7—13 years, who were members of a boarding school in Calcutta. The characteristic symptoms of the disease and the morphology and cultural characters of the fungus are described in detail. GHOSH & MAPLESTONE (1941) described a simple method of impregnating culture media with gentian violet which proved effective in preventing growth of bacteria while it allowed free growth of ringworm fungi of the skin. GOVINDAN NAIR (1942) showed from the statistics of four years from 1937—40 from King George's Hospital Vizagapatam that the ringworm disease caused by *Epidermophyton inguinale* occupies the third place among skin diseases affecting all the parts of skin such as face, trunk, limbs, the gluteal and inguinal regions, the palms, soles and nails of both fingers and toes. He did not come across even a single ringworm of scalp as the etiological agent is a fungus other than *E. inguinale*, which apparently was absent in those parts.

Although only three cases of *Tinea imbricata* had been reported before 1942 (two by CASTELLINI from south India and one by ACTON & GHOSH from Bengal), DEY & MAPLESTONE (1942) drew attention to the common occurrence of *Tinea imbricata* in India, on the basis of extensive survey of DEY who noticed a large number of typical infections among the aboriginal tribes in Assam. DEY collected scales from patients from different villages and subjected these materials to laboratory examinations isolating *Endodermophyton tropicale* CASTELLANI from two and *Endodermophyton indicum* CASTELLANI from three patients rest being unsuccessful. These two species on subculturing ultimately proved to be indistinguishable which confirmed the view expressed by ACTON & GHOSH (1934) that *E. tropicale* and *E. indicum* were probably identical. The authors have further discussed the taxonomical position of the genus *Endodermophyton* and considered the differentiation of the genus *Achorion* and *Endodermophyton* as unjustified and thought that eventually *Achorion concentricum* BLANCHARD 1896 (GUIRAT & GRIGORAKI 1928) will be adopted as the correct name of the species.

GHOSH, DEY & PANJA (1947) recorded the incidence of *Trichophyton gypseum* which caused small spored *Ectothrix trichophyton* infection in man. This species causes *Tinea capitis* (ringworm of the scalp) both in children and adults but in the latter it also causes *Sycosis barbae*. They studied seven isolates of the fungus and found pleomorphic changes in culture soon after isolation.

GHOSH, PANJA & DEY (1948) investigated a large number of infected nails and grouped them under two headings, (a) *Onychia* and (b) *Perionychia* and reported the preponderance of *Trichophyton purpureum* (*Epidermophyton rubrum*) over *Epidermophyton floccosum* in *Onychia* and the preponderence of *Monilia sp.* in *Perionychia*. GHOSH, PANJA & DEY (1948a) surveyed the ringworm fungi of glabrous skin in and around Calcutta. Their clinical diagnosis was confirmed by microscopic and cultural findings, for the former freshly prepared sodium sulphite solution was used for its better cleaning and keratolytic properties in preference to KOH solution. *Epidermophyton rubrum* was found to be more common than *Epidermophyton floccosum*. They also pointed out that each country has its characteristic fungus causing the ringworm of the glabrous skin, e.g. *Epidermophyton floccosum* in U.K., *Trichophyton gypseum* in U.S.A. and *Trichophyton purpureum* (*Epidermophyton rubrum*) in China.

GHOSH (1948) statistically analysed 50,000 skin diseases as corded in the outpatient department of the School of Tropical

Medicine during 1942 to 1946 and drew the conclusion that (a) scabies is the most common disease encountered, (b) next in occurrence is fungus infections. Among the latter the ringworm of the body is much more common, followed by ringworm of scalp and least being *Monilia* infection. Further, (c) ringworm of the scalp due to *Microsporon* is scarce in the plains of India . It is mostly imported from hill stations by Anglo-Indian, European and Jewish School children, the disease when found in Indian children proved to be due to *Trichophyton violaceum*. Among the Nepali (Gurkha) adults the disease was caused by *Trichophyton gypseum* and this was interpreted as racial characteristics. Favus was not represented among the recorded cases according to the author. The disease commonly occurs in Kashmir and North Punjab but it is significantly absent in the plains of India excepting the desert areas of Rajputana and Sind. On the basis of 215 cases of ringworm of hair DEY (1953) classified the disease under three headings, (i) ringworm of the scalp (*Tinea tonsurans*), (ii) ringworm of the beard(*Tinea barbae*), and (iii) *Lanungo* hair. Of these ringworm of scalp is particularly common.

The fungi causing the disease are arranged by him in descending order of their frequencey as *Microsporon Audouinii*, *Trichophyton violaceum*, *Microsporon ferrugineum*, *Trichophyton crateriforme*, *Trichophyton gypseum*, *Trichophyton violaceum* var. *indicum* and *Achorion Actoni*.

CHAKRABORTY, BANERJEE & GHOSH (1953) reported a rare variety of ringworm infection of the body with typical lesions presenting considerable difficulty in the diagnosis. The causal fungus was identified as *Trichophyton discoides*.

CHAKRABORTY, GHOSH & BANERJEE (1953, 1954) reviewing the skin diseases in animals communicable to man concluded that approximately 27 % of all the dermatophytosis are due to pathogens parasitic on lower animals, ringworm of scalp being the most important and widely distributed among the diseases contacted through domestic animals. The treatment of these anthropophilic pathogens is simple but their control presents certain difficulties. *Microsporon lanosum* is more common from animal sources than *Microsporon Audouinii* infection. DEY (1953a) reviewed infections due to *Trichophyton purpureum* causing ringworm of the glabrous skin with extensive lesions of the body particularly of the trunk and discussed the diagnosis and treatment of disease together with the cultural characters of the pathogen. He also concluded that this type of ringworm is fairly common and in 1941 alone 1218 out of 11,300 new cases in School of Tropical Medicine had this infection.

DEY & KAKOTI (1955) reported for the first time the incidence of *M. gypseum* in India from a laboratory bred rabbit with a favic lesion on the skin over the nose and maxillary region. The presence of the fungus in soil from the vicinity of the animal house was also confirmed. BARAT (1955) listed the skin diseases encountered by him amongst children in rural area at Amargarh, U.P. and arranged them in the following descending order of their incidence (1) Eczema (2) Scabies (3) Ringworm (4) Impetigo (5) *Pediculosis capitis* (6) Sweat rash (7) Urticaria. The diagnosis were based on clinical examination only.

ANDLEIGH (1955) studied the efficacy of different clearing techniques using materials from 122 superficial fungus infections and found the old time honoured alkali clearing method to be best yielding highest positive result. Periodic acid Schiff's stain method was almost as good as KOH but was not so simple.

PANJA (1956) described the disease and the general characteristics of trichophytide and noted the absence of fungus in the phytide lesions. He suggested the possibility of trichophytide in all frank eczema of hands and feet. He doubted the diagnostic value of trich ophytin test in all cases and stressed the importance of microscopic examination of eczematous skin lesions.

DEY & KAKOTI (1956) for the first time reported a *faviforme-ectothrix trichophytosis* caused by *Ectotrichophyton discoides.*

BANERJEE (1957) reviewed the common skin diseases including mycotic infection of the hands encountered in the tropics giving their symptoms with illustrative photographs. DEY (1957) in his report on the superficial *pustular folliculitis* including *Sycosis barbae* described the symptoms, etiology, diagnosis and treatment and specially the results of the treatment with Aureomycin hydrochloride.

SRIVASTAVA (1957) reported *Trichosporon cutaneum,* as the etiological agent for the infection of the interdigital skin of the foot of a man, for the first time.

BEHL & SHARMA (1958) in their survey of mycotic infection in Delhi observed that dermatomycoses are relatively common being represented by *T. pedis, T. cruris, T. corporis,* and *T. unguium.* They found *T. mentagrophytes* to be the most common etiological agent for the disease. Out of 138 cases subjected to mycological study 50.7 % showed positive fungal evidence on microscopic examination whereas 49.3 % failed to do so. *T. mentagrophytes* was found in 47.3 % of the cultures, followed by *T. rubrum* and *T. violaceum* in 26.3 % and 21 %, respectively, the least being *T. sulphureum* found only in 5.2 % of the cultures. Among the various diseases encountered *Tinea capitis* was rare there being only 6—7 cases in a year out of a total of 14 thousand cases in Delhi. Therapeutic response to Penicillin, iodides, sulphonamides or Chloramphenicol was poor.

MITRA (1958) recorded three *Kerion* infections in prepubetral age, all being *Microsporon* infection from animal source. *M. gypseum* and *M. canis* were isolated from one and two patients, respectively, as the etiological agent. Treatment with Multifunganin was useful. R. K. PANJA (1958) came across a fúngal infection (*Sycosis barbae*) in Calcutta which he described along with clinical symptoms and other relevant features. D. PANJA (1958) in his paper on 'cheiro and podopompholyx' described the pompholyx disease and its allied varieties in details with etiology, clinical manifestation and treatment etc. G. PANJA (1958) in a separate communication in 1958 discussed the clinical features of etiology, cultural and mycological details, diagnosis and treatment with prophylaxis of *Tinea cruris* and *Tinea circinata*.

LAHIRI (1958) reported a *favus* infection of the scalp, forehead and nape of neck, by *Trichophyton Schoenleinii* which is a rare disease in Bengal. In follow up study of 24,500 fungal diseases during the course of five years this proved to be only one with *T. Schoenleinii* infection. During the same period LAHIRI (1958a) investigated 11515 instances of *tinea pedis* and found that 65 % was due to *T. rubrum*, showing its increase under tropical conditions. Certain relation between the complication of fungus infection with the nutritional factors, particularly vitamin A and C, was shown. LAHIRI, SEN, KUNDU & LAHIRI (1958) in an analysis of 24,500 fungal infections encountered *tinea versicolor* in 7000 cases the majority being associated with other skin diseases. The incidence varied with the season and was maximum in summer. MAJUMDAR (1958) discussed in detail the concept of 'id' reaction along with its etiological studies and clinical picture and suggested methods of treatment. ANDLEIGH (1958) tried various mediums but failed in his attempt to find an easier method to culture *Malassezia furfur*.

SRIVASTAVA & GUPTA (1958) in a survey of human skin fungi in Lucknow found *T. rubrum* to be the most dominant pathogen (23.1 %). The other pathogens, viz., *T. mentagrophytes*, yeast-like fungi and *M. Audouinii* were less common being 14.4 %, 15.6 % and 0.7 %, respectively. Species of *Aspergillus*, *Alternaria*, *Rhizoctonia* and other unidentified fungi were encountered as contaminants.

BANERJEE, RAO & CHAKRABORTY (1958) studied the ringworm diseases of man at the School of Tropical Medicine during 1955—57, and found the prevalence of ringworm of glabrous skin. They successfully isolated 132 strains of *T. rubrum* and one strain of *E. floccosum*, the former being the most common skin disease fungus.

MUKHERJEE, CHAUDHURI & RAY (1958) described a modified staining technique to demonstrate the ringworm fungus in skin and nail scrapings. They found that their pretreatment with KOH solutions and subsequent staining with periodic acid Schiff method helps the mycelial filament to take a deeper stain, revealing the presence of ringworm fungus even if the infective agent is very scanty. The same authors (1958a) reported a new method of staining parasitic fungi in skin and nail lesions by utilising M. R. Solution. Treated with this the spores and hyphae of fungi causing ringworm, *tinea versicolor* (*Malassezia furfur*) and bottle bacilli

(*Pityrosporum ovale*) of dandruff are all stained in different shades of red. The arthospores within the hyphae remained unstained. The nuclei within the structures were clearly visible as small rings with karyosomes.

DEY (1959) listed all the Indian species of fungi pathogenic to man and animals. Guided chiefly by the International Rules of Botanical Nomenclature he suggested a new nomenclature to be used with parallel synonyms along with it.

BANERJEE, RAO & CHAKRABORTY (1959) found dermatomycoses to be the most common mycotic infection in this country. A definite change in the relative abundance of the pathogens from those of the previous findings was observed. *T. rubrum* was the predominant agent for the etiology of ringworm. Infrequent appearance of *tinea capitis*, conspicuously high incidence of *tinea circinata* and the varied clinical pictures with chronicity and extensive involument of glabrous skin of dermatomycoses were some of the noteworthy points. DESAI, MARQUIS & BHATT (1959) reported the results of their studies of 624 superficial mycotic cases at the skin department K. E. M. Hospital, Bombay. *T. rubrum* was found to be the most common and therapeutically resistant pathogen. DEVI & DHANVANTARI (1959) in a general discussion on the fungal diseases in man have divided it into two groups (a) superficial, (b) deep seated. Pointing out the difficulties in the classification of these pathogens they suggested that the classification should be based on the taxonomic position of the causative fungi following the normal mycological usage. They have further stressed the need of systematic study of the mycotic fungi and their ecology for correct diagnosis of the disease and their treatment. DEY (1959a) also has suggested a natural classification of mycotic diseases.

DEY (1959b) presented a survey of 198 cases of ringworm of the scalp, studied over a period of 20 years. He found the disease to be uncommon in India and occurring mostly in European or Anglo-Indian children coming from hill stations. The pathogens isolated from these being *M. Audouinii*, *T. ferrugineum*, *T. violaceum*, *T. gypseum*, *T. verrucosum* and *M. canis*, the dominant ones among these being *M. Audouinii* and *T. violaceum*. G. PANJA (1959) described the method adopted by him to succesfully culture *Malassezia furfur* on an egg medium in the drier part of the slopes in the form of white chalky colonies visible to naked eye. Morphologically and culturally they were found to belong to the genus *Malassezia*. Cocoanut oil was found to enhance the growth in form of copious, moist and cheesy colonies. G. PANJA (1959a) discussed the justification of the name of the disease *Seborrhoeic dermatitis*. Role of *Pityrosporum ovale*, its relation to sebaceous glands and the treatment with sulphur in regard to the disease were also elucidated. The same author (1959b) described the mode of examination, microscopic and cultural determination of some unusual and interesting ringworm pathogens infecting glabrous skin. He also described the differential diagnosis and his own line of treatment. VERMA (1959) investigated 125 cases of *tinea versicolor* (obtained within seven months) at S. S. G. Hospitals, Baroda. He gave a short account of the literature of *tinea versicolor* and discussed the unusual features of his findings suggesting that the disease presents a little different picture in tropics than what is usually mentioned in the literature.

GOKHALE, THIRUMALACHAR & PADHYE (1959) examined 200 cases at Poona out of which 83 were positive for fungus infection. The organisms were observed microscopically or grown in culture to establish the diagnosis. The fungi successfully isolated were *Actinomyces bovis, Candida albicans, T. tonsurans, T. mentagrophytes, M. Audouinii, Malassezia furfur, Epidermophyton* sp. and *Hormodendron.*

DAS-GUPTA & SHOME (1959) studied 405 suspected and diagnosed mycotic infections during the years 1955—57 out of which 306 were dermatomycoses, 36 *tinea versicolor,* 30 candidiasis and 21 erythrasma cases. In dermatomycoses, two genera *Trichophyton* and *Epidermophyton* were the etiological agents, there being a complete absence of the genus *Microsporon. Trichophytons* were represented by four species, *T. rubrum, T. mentagrophytes, T. tonsurans* and *T. verrucosum* and *Epidermophyton* by one species *E. floccosum. T. verrucosum* is a new record from India. *Candida albicans* was the only pathogen isolated from Candidiasis. *Tinea versicolor* and erythrasma had *Malassezia* and *Microsporon* as their causative organisms. *T. rubrum* was found to be responsible for 50 % of the diseases, *T. mentagrophytes* for 26 %, *T. verrucosum* and *T. tonsurans* for 8 % and 5 %, respectively. *Epidermophyton floccosum* and *Candida albicans* were responsible for 3 % and 8 % of the diseases, respectively.

GUPTA & SHOME (1959) in their survey of dermatomycoses in Uttar Pradesh during 1954—58 analysed 620 cases of which 246 were *tinea corporis,* 221 *tinea cruris,* 97 *tinea pedis,* 24 *tinea barbae,* 20 *tinea capitis* and 12 *tinea unguium.* They found dermatomycoses to be dominant among adults (60.32 %), less prevalent among boys and girls attaining the age of puberty (33.23 %) and comparatively rare among children (6.45 %), the disease was found to be more common among men than women, ratio of the sexes being 3 : 1. Dermatophytes in the province were represented by two genera, i.e., *Trichophyton* and *Epidermophyton,* the former showing the overwhelming majority of 98.08 %. *T. rubrum* was the most prevalent species of the dermatophytes being represented by 56.73%. The other pathogens, e.g., *T. mentagrophytes* and *E. floccosum* were 31.73 % and 1.92 % respectively. Besides the dermatomycoses fourteen cases of 'id-eruption' were studied and discussed along with their clinical symptoms.

SHOME (1959) recorded from Lucknow twelve cases of ringworm of glabrous skin with *T. verrucosum* as the etiological agent for the first time in India. The characteristic symptoms of the disease and the indentification of the pathogen on the basis of morphological and cultural characters on different nutritive mediums were discussed. SHOME (1959a) in his survey of ringworm of scalp in Uttar Pradesh found the disease to be very uncommon being only 3.2 % of those suffering from dermatomycoses. He came across altogether twenty cases of which 18 (90 %) were among boys and girls below sixteen. The unusual features were, the prevalence of the disease among Indian children, the complete absence of the pathogen *Microsporon Audouinii* and the occurence of the disease among the inhabitants of the plains. The etiological agent of ringworm infection of scalp were *T. tonsurans*, *T. rubrum* and *C. albicans*, the overwhelming majority being due to *T. tonsurans*. The isolation of *T. rubrum* and *C. albicans* as the causative pathogens for ringworm of scalp are new reports from India.

B. Therapeutics

Simultaneously with the study of the incidence and distribution of dermatomycetes there have been investigations to find drugs, chemotherapeutic and antibiotic agents to combat and control the mycotic diseases.

DEY & GHOSH (1945) recorded the use of gonadotropic hormones in the treatment of ringworm of scalp with satisfactory result. Among these *Microsporon Audouinii* and *Trichophyton crateriforme* infections responded better than *Trichophyton violaceum* infections. Female children responded better than male children. Hormone therapy together with local antiseptic treatment gave better results than hormone therapy alone.

GHOSH & PANJA (1946) tested several drugs as prophylactics against ringworm fungi and recommended the use of 4 % solution of glacial acetic acid and 25 % alcohol which was found to be effective in preventing ringworm infection.

MAJUMDAR & BOSE (1955, 1955a) in their studies with the antifungal microorganisms from Indian vegetables, fruits and soils isolated numerous strains of Bacteria (*B. subtilis*), *Actinomyces*, *Penicillium* and other moulds showing antifungal activities against different pathogenic and non-pathogenic fungi including the dermatophytes, viz., *T. rubrum*, *T. sulphureum*, *M. Audouinii*, *C. albicans*, *A. Schoenleinii* and *Nocardia* sp. The work was further extended by DUTTA, MAJUMDAR & BOSE (1955) who reported the isolation of 119 antagonists out of a total of 230 microorganisms from similar sources, screened for their antifungal properties against human pathogenic fungi, viz., *T. rubrum*, *T. Schoenleinii*, *T. sulphureum*, *M. Audouinii*, *Nocardia* sp. and *C. albicans*. Most of the antagonists were *Bacteria* (*B. subtilis*) and the rest being *Penicillia*, *Aspergilli* and *Streptomyces*.

MAJUMDAR, SEN GUPTA, DAS GUPTA & CHAKRABARTY (1956) reported their results of the treatment of pruritic skin diseases with Crotonyl-n-ethyl-o-toluedine cream. They tried it on 323 patients; in 87 % the result was satisfactory, in 20 % the improvement was rapid whereas in 13 % (which included sycosis, *pityriasis versicolor*, etc.) there was no improvement.

SRIVASTAVA (1957) isolated *Trichosporon cutaneum* from the interdigital skin of the toe of a man which was non-pathogenic to laboratory animals. In the *in-vitro* experiments the fungus was susceptible to undecylenic acid but to a less extent to nycil and quinone compounds.

RAY (1958) put forth suggestions for the treatment of ringworm of scalp (*tinea tonsurans*) by X-ray based on the clinical tests. An interesting point observed by him was the spontaneous cure of the disease at puberty.

GUPTA & BASU (1958) in their studies of the treatment of fungal infection and psoriasis with Bradex-vioform cream found that this cream can safely be recommended as a valuable drug for chronic psoriasis as well as various types of mycotic skin diseases. MITRA & BANERJI (1958) on further work to evaluate Bradex-vioform as an antimycotic drug found it to be definitely efficacious in all infections due to *Epidermophyton floccosum* and *Trichophyton rubrum* whereas in others its effect is much less. BEHL & KUMAR (1958), also in trial treatment of 50 mycotic skin diseases with Bradex-vioform found 90 % of very good to moderate results. The results are in agreement with those mentioned above.

CHUKERBUTTY (1958) in his clinical trial of Multifungnin in all its commercial forms found in 20 cases of dermatophytosis very encouraging results, the fungistatic and antipruritic property being quite evident. However more trials are needed to establish the specificity of this drug.

SRIVASTAVA & GUPTA (1958) tried 31 asymmetrical diaryl sulphones, sulphoxides and sulphides *in vitro* for their activity against *T. rubrum*. The sulphides were found superior to Nycil but somehow less active as compared to undecylenic acid.

SRIVASTAVA (1959), in his studies of the antifungal activity of some sulphur containing compounds, screened the antifungal activity of a copper chelate of a sulphone hydrazide, two sulphoxides three sulphides and a potassium salt of benzyl iso-thiocyanate sulphonic acid against the dermatophytes (*T. rubrum, T. sulfureum, T. mentagrophytes, M. Audouinii*, and *M. gypseum*) and 3 yeast-like fungi (*C. albicans, Geotrichum* sp. and *Trichosporon cutaneum*) using undecylenic acid as the standard. The potassium salt of benzyliso-thiocyanate sulphonic acid completely inhibited the growth of all the fungi except *M. Audouinii*. It appears to be superior to undecylenic acid against several fungi but not against *M. Audouinii*, p-methyl amino-p-amino diphenyl and p-ethyl amino-p-amino diphenyl sulphides completely inhibited the growth of *T. rubrum, T. sulfureum, T. mentagrophytes, Geotrichum* sp. and

Trichosporon cutaneum at certain concentrations but had little or no effect on *M. Audouinii*, *M. gypseum* and *C. albicans*. Di-aminodiphenyl sulphide and the sulphoxides were able to stop the growth of *T. rubrum*, *T. sulfureum* and *T. mentagrophytes* only at the highest concentration used. Copper chelate of sulphone hydrazide did not show much activity.

Banerjee, Majumdar & Bose (1959) reported Mycobacillin to be a broad spectrum antifungal antibiotic active against skin pathogens, phytopathogens and many rice spoilage fungi. Its minimum inhibiting concentration in most of these was 20/mcg/ml, although it goes up to 50/mcg/ml in case of one strain of *Candida albicans*.

G. Panja (1959c) discussed the therapeutic problem of the dermatomycoses in India along with the results of different fungicidal drugs tried by him on large number of ringworm diseases.

C. Physiology

The pathogens responsible for superficial mycoses are characterised by their specialisation in parasitism on keratinised tissues of man and animal, although the taxonomically related species of these fungi are either plant pathogens or saprophytes. The fact that the knowledge of physiology of the parasites is the first requisite for intelligent approach towards the eradication of the disease has led to the study of physiology of these fungi, dealing with their nutritive requirements, including micro-nutrients and vitamins, and also the enzyme system present in the organisms. These studies can give a better insight into the mode of infection, host-parasite relationship and their very specialised nature of parasitism. The work along this line here is few and very recent.

The study of the enzyme system in dermatophytes are being carried out in only two laboratories, (1) The School of Tropical Medicine and (2) The Mycology laboratory of Lucknow University. The former generally used the histochemical methods while the biochemical method is being used by the latter.

Banerjee, Chakraborty, Ray & Rao (1956) published their results of the experiments on the localisation of alkaline phosphate in certain skin diseases. In the same year Chakraborty & Banerjee (1956) communicated the results of their experiments on Isoniazid and para-amino salicylic acid in cutaneous nocardiosis. Banerjee, Ray, Ghosh & Chakraborty (1957) working on the nuclear reaction in *Trichophyton rubrum*, reported the presence of DNA in fungal nucleus and hyphae by Feulgen reaction.

Bhattacharya, Ray & Chakraborty (1958) reported the results of their further observations on the site of maximum alkaline phosphate activity in *Trichophyton rubrum*. Among the different parts of the fungus, nucleus gave the strongest reaction of the enzyme. Some macroconidia showed deposit of cobalt sulphide in the whole of cytoplasm and some at two poles only. Interhyphal septum was conspicuous by the absence of enzyme. In the older hyphae the walls showed heavier deposit of cobalt sulphide in granular form than the cytoplasm. Cytochemical tests also revealed the presence of calcium, iron and lipids in this fungus. In a separate paper the same authors (1958a) gave an account of the techniques followed and the result of their observations on the distributions of certain cytochemical entities in *Trichophyton rubrum*. The fungus was found to have a rather complex cytochemical make up, containing iron, calcium and lipoid besides other cell constituents.

BHATTACHARYA, RAY & BANERJEE (1959) communicated in a brief note the results of the study of two enzymes lipase and succinic dehydrogenase in some dermatophytes and affected skin. Reaction for lipase was more intense in *M. gypseum* than in *T. rubrum*. Extensive reaction was visible in the core than in the periphery of the fungus. In macroconidia the enzyme in the cytoplasm was in the form of scattered granules. Succinic dehydrogenase was present both in the hyphae and macroconidia which appeared either blue (diformazan) or red (monoformazan) granules in the cytoplasm. Normal and diseased skin showed no reaction for lipase. The diseased skin showed more intense reaction for succinic dehydrogenase as compared to the normal. Monoformazan crystals were visible in the cytoplasm of the basal and prickle cell layer only in the diseased tissue.

The School of Mycology Lucknow University has long been engaged in the study of physiology of phytopathogenic fungi. In recent years the scope has been widened to include the physiology of dermatophytes. SHOME (1959b—g) has studied the physiology of *T. rubrum*, *T. mentagrophytes* and *Epidermophyton floccosum*. His investigations (1959b) on the pigment production by *T. rubrum* showed that both carbon and nitrogen sources were essential and the pigment was produced in 11 carbon sources but not in presence of lactose. Very poor yellow to brownish pigmentation appeared in presence of different amino acids except in presence of asparagine and leucine where the pigment was characteristic deep purple. Sodium chloride, even up to the dilution of 0.25 g per litre of the medium, helped to intensify the colour from purple to dark brown. pH of the medium had no effect on pigmentation. The pigment was easily soluble in dilute acid solution or chloroform giving an yellow colour in the former and changed to reddish brown in alkaline base; the change was reversible.

SHOME (1959c) studied the extensive variability in case of single spore cultures of *T. rubrum*, *T. mentagrophytes* and *E. floccosum* in light of the dual phenomenon in dermatophytes put forth by WILHELM and held that this cannot be adequately explained by WILHELM's concept of dual phenomenon. SHOME (1959d) isolated an asporogenous strain of *Bacillus subtilis* producing at pH6.5-7.5, extra-cellular, thermostable antibiotic which has specific fungistatic action against *T. rubrum, T. mentagrophytes* and *E. floccosum* and no effect on the phytopathogenic or saprophytic fungi tried. The hyphal tips when in contact with antibiotics curves inwards but no *in-vivo* test was made.

The investigations of Das Gupta & Shome (1959a) on the effect of different carbon, nitrogen and vitamin sources on the growth and sporulation is the first of its kind from India dealing with the nutritional physiology of the three dermatophytes *T. rubrum, T. mentagrophytes* and *E. floccosum.* Of the 12 carbon sources employed glucose and fructose followed closely by sucrose supported best growth whereas ribose and rhamnose failed to support any growth for the dermatophytes, the rest of them being moderate to poor supporters for either growth or sporulation.

So far as nitrogen sources are concerned none of the three dermatophytes were able to utilise any of the inorganic nitrogen supplied. Among the organic sources complex forms of peptone and casein hydrolysate proved to be best for the growth and sporulation. Out of 14 amino acids tried as nitrogen source the dermatophytes failed to utilise glutamic acid, tryptophane and histidine; this showed fairly good growth in leucine, arginine, asparagine, glycine, and lysine; the rest supporting moderate to poor growth. Out of the six different combinations of amino acids tried, alanine + arginine + leucine and leucine + arginine + asparagine, supported best growth but comparatively complex organic compounds proved to be the best source for nitrogen.

The dermatophytes studied did not show deficiency for the ten vitamins tried. It was found, however, that the fungi grow better when these vitamins were added to the mediums.

Shome has so far employed only biochemical methods for the study of enzyme systems of the dermatophytes. The estimations have been both qualitative and quantitative. In *T. rubrum* and *T. mentagrophytes* Shome (1959e) found lipase, amylase, invertase, raffinase, cellulase, pectinase, proteolytic enzyme, urease, rennatase, laccase, oxidase, peroxidase, catalase, deaminases, phosphorylase, dehydrogenase and phosphatases. Of these catalase was present both intra and extracellularly in *T. mentagrophytes* whereas in *T. rubrum* it was only intracellular. Butyrase and tyrosinase were absent in both the micro-organisms.

A detailed study of the deaminase, phosphorylase and dehydrogenase activities of these two dermatophytes has shown that the deaminase activity was negligible to very poor in presence of dl-serine and l-glutamic acid whereas it was appreciably good in presence of l-leucine, l-asparagine dl-alanine, dl-asparagine closely followed by dl-aspartic acid. The results are in general agreement with the findings of the nutritional properties of these fungi in presence of individual amino acids (Shome & Verma 1959).

Shome (1959f) studied the dehydrogenase system primarily involved in tricarboxylic acid cycle (Krebs cycle), viz., isocitric, d-ketoglutaric, succinic, and malic of *T. rubrum* and *T. mentagrophytes*, the former pathogen showing a definitely higher dehydrogenase activity. Appreciable dehydrogenase activity was found in all the four acids indicating that tricarboxylic acid cycle plays an important role in their metabolism and these microorganisms draw energy through this cycle.

The study of phosphorylase activity in these pathogens showed the appreciable presence of this enzyme, which catalyses the decomposition of polysaccharide into glucose-l-phosphate. As the reaction is reversible, the activity of the enzyme was measured under conditions when glucose of glucose- l-phosphate was attached to the primer starch liberating phosphorus. This has been taken to indicate that the enzymic system for the breakdown of starch into glucose-l-phosphate which may be utilised through the Embden, Meyerhoff and Parnas cycle in respiration, operates in these pathogens. The enzymic activity was, however, of greater intensity in *T. rubrum* than in *T. mentagrophytes* (SHOME 1959g).

On the basis of above enzymic studies the authors believe that there appears to be a definite relationship between the pathogenicity and activities of various enzymic systems present in the dermatophytes studied.

ACTINOMYCOSIS

It is an acute or chronic fungus infection of man attacking mainly the connective tissues of cervico-facial region, the thoracic and abdominal viscera.

T. SUR (1918) commented on the absence of any reported actinomycosis in India prior to 1918 when he started investigation in this field and claimed to be the first to report three cases of actinomycosis due to *Actinomyces hominis* whose morphological and cultural characteristics were also studied. The same year (1918) SAHA reported an instance of actinomycosis caused by *A. pleuromediastinitis* in an hindu male of 22 years. Case history, external symptoms and the blood report failed to reveal the true nature (etiology) of the disease which was only diagnosed by the greenish granules observed after operation and microscopic examination of the pathogenic fungus.

ACTON & MCGUIRE (1930) isolated for the first time *Actinomyces keratolytica* from the infected palms and soles of 8 bare-footed persons causing Bengal rot or Mango toe or foot teethers. The disease in Bengal is known as *Haja* (mango toe), *Phata* (cracked heels and *Chalune* (pitted soles). Subsequently in 1931 they were able to demonstrate the lesions in every case by the use of MCGUIRE's stain for diphtheria. They also overcame the difficulty of successfully cultivating the organism with certainty by culturing it in Norris medium.

BASU (1937) published an account of a new strain of *Actinomyces* obtained by blood culture. He named it *Actinomyces sanguinus* n. sp. and described in detail its morphological and cultural properties. G. PANJA (1948) reported an unusual actinomycosis on the left cheek of a patient over the molar prominence showing reddish painful swelling with softening in the centre. Microscopical findings and cultural examination confirmed the disease to be actinomycotic. *Actinomyces* isolated was regarded as a new species. The patient improved with potassium iodide but there was relapse within three months. It was completely cured by scraping and X-Ray exposure. ANDLEIGH (1951) reported an actinomycosis of the left palm of a hindu female of 40 years. Cultural examination is lacking and the specific identification of the fungus was not made.

SEN (1951) examined an actinomycosis of the lung, the microscopical examination of the sputum and the granules discharged through the sinus in the chest wall showed the infective organism. SUBRAMANIAM (1952) reported a similar actinomycosis of the lung. Sputum showed actinomycotic granules while pus revealed *Actinomyces* but culture was sterile. RANAVRE (1951) identified a primary actinomycosis by the histological examination of the tumor-like mass of the chest wall of the patient. He thereby concluded that all chronic and obscure lung affections may be investigated for mycotic infections.

BASU (1952) reported 5 cases of actinomycosis. The identity was established by isolating the causal organism from different lesions confirmed by cultural findings. He found that primary cultures take time to grow, sometimes as long as a month. ANDLEIGH (1955) described an actinomycosis caused by *Actinomyces bovis* which exhibits some interesting features. After due experiments he suggested the use of Streptomycin against the actinomycotic infection. PANJA (1956) reported an interesting case of *Actinomyces madurae* showing typical sinuses and granules on an unusual site, viz., the abdominal wall near the umbilicus. The pathogen was successfully isolated but the treatment failed to cure the disease.

BANERJEE (1958) in his survey of primary actinomycosis of the skin for the last five years came across only 15 instances of actinomycosis out of total of 70,000 cases at the Skin Department of Medical College Calcutta. Treatment of this disease still presents a problem.

CANDIDIASIS

Candidiasis is a fungus disease caused by the members of the genus *Candida* which may produce lesions in mouth, skin, nail, vagina, lungs, bronchi and occasionally cause meningitis, endocarditis or septicema. The infection has been found to be of a localised as well as generalised nature in relation to human body.

General

KUMAR (1925) & SHELMIRE (1925), each independently, recorded *Monilia* from infection of nails. MAPLESTONE & DEY (1939) recorded the incidence of *Monilia* and *Saccharomyces* which were more commonly found on palms than in infections of the foot.

BATT & SHETY (1946) recorded the occurrence of *Monilia albicans* in dental caries not reported before from tooth.

PANJA & BANERJEE (1950) studied 35 cases of *perionychia.* Microscopical study of the direct smear did not reveal the causative organism but in 23 out of 35 *Monilia* could be cultured. The species of *Monilia* identified were *Monilia pinoyi, Monilia nabarroi* and *Monilia bronchialis*, of which *Monilia pinoyi* was commonest in India while *Monilia albicans* is most common in other countries. In India this disease is more common in females than in males. It is prevalent in labourers, cultivators, gardeners, housewives, and amongst those that deal with sugar candy, vegetables and fruits.

SINGH (1950) reported 3 cases of pulmonary moniliasis from which *Candida albicans* was isolated as the causal organism. In two he found great similarity in signs, symptoms and roentgen findings between pulmonary moniliasis and pulmonary tuberculosis. He emphasized the importance of proper care in diagnosis, which should never be based on roentgenological findings alone but should be supported by laboratory procedure and other examinations.

BANERJEE & GUPTA (1956) investigated an acute respiratory infection by *Candida albicans* with a discussion on the role of bacterial and fungal infection of this type.

BANERJEE (1958) gave a brief description of the manifestation of moniliasis. He also reports an unusual case of deep dermal and subcutaneous moniliasis, the first record of this kind of monilial disease from India and perhaps from abroad as well.

KASLIWAL & ANDLEIGH (1958) reported a case of bronchopulmonary moniliasis with detailed pathological tests and successful isolation of *Candida albicans* in culture. Bronchopulmonary moniliasis is sufficiently rare and interesting to warrant attention. SRIVASTAVA, GUPTA & MUKERJEE (1958) studied the morphology and physiology of four strains of yeasts from human faeces and their *in vitro* sensitively to Nystatin. They found that the four strains could be divided into three groups in regard to their morphological and physiological characters as well as their sensitivity to Nystatin. Two strains were found to be similar to *Candida albicans* while the other two differed from it and also from each other.

SHOME (1959) reported candidiasis of scalp for the first time from India. The characteristic symptoms of the disease along with the procedure for identification of pathogen *Candida albicans* by fermentation and growth reaction has been described in detail.

BASU, MAJUMDAR, RAO & BANERJEE (1959) studied a series of 80 cases of candidiasis and found a significant correlation between the incidence of *C. albicans* with the presence of similar yeast-like organism in stool and oral cavity. CHAKRABORTY, RANDHWA, ROY & VISHWANATHAN (1959) examined 89 throat swab culture from normal population and 84 samples from patients suffering from bronchopulmonary diseases like chronic bronchitis, asthma, pneumonia, lung abcess, tuberculosis etc. 34.8 % positive culture of *Candida* species was obtained in throat swab of normal people whereas 38.1 % positive culture of *Candida* species in case of bronchopulmonary cases.

Desai, Marquis & Bhatt (1959) studied 46 cases of vulvovaginitis including relapses. 27 cases of *C. albicans*, 5 of *C. tropicalis* and one each of *C. stellatoidea*, *C. Krusei* and *C. pseudotropicalis* were successfully indentified on basis of laboratory investigations. Dey (1959) reported two cases of pulmonary moniliasis caused by *C. albicans* in the vaginal discharge of pregnant women selected at random. He found the incidence of moniliasis to be much higher than *Trichomonas vaginalis*, the majority belonging to women between 20 to 30 years of age. 92 % of positive cases were found among the multiparous group. The details of symptomatology has been discussed by him in light of cultural report.

Pohowalla & Kaul (1959) studied 500 newborns and their mothers to determine the clinical and subclinical oral monialiasis in the newborns in relation to vaginal moniliasis in the mothers. No definite correlation was found to exist nor could the incidence be correlated with the mode of delivery. The predominant strains of monilia found in the newborn in the studies were *C. albicans* and *C. tropicalis*.

Purandare & Narvekar (1959) recorded the incidence of fungus infection of the female genital tract in nonpregnant patients complaining of leucorrhoea. The relative incidence of fungus infection was found to be much lower than that reported from abroad but higher than the previous Indian reports. No special preference for any single age group was found. An interesting point was the absence of macroscopically demonstrable pathological lesion in 48 % of positive cases. In other 15 % the isolated fungi did not belong to the *Candida* group. The authors concluded that except for a few showing typical clinical picture of fungus infection, only cultural methods can be relied upon for the final diagnosis.

For the study of the incidence of vaginal moniliasis Satyavathi (1959) examined 400 women attending the antenatal clinic. Of the 120 normal pregnant women subjected to mycological study 54 showed positive culture giving 45 % incidence of non-symptomatic *Candida* in normal pregnant women. 76 of the 400 patients were symptomatic giving an incidence of 19 %. Co-existence of *Monilia* and *Trichomonas* was observed in 12 cases. Daftary (1959) studied the incidence of monilial and trichomonad infection in 149 pregnant women. The incidence of only monilial infection was found to be more in the asymptomatic group than the symptomatic group.

Therapeutics

Maplestone & Dey (1939a) recommended the use of dyes (1 %

gention violet and brilliant green in 10 % alcohol solution thrice daily) for the cure of *purulent folliculitis* due to *Achorion* and interdigital infection due to *Epidermophyton floccosum*, *Actinomyces keratelytica*, *Monilia* and *Saccharomyces*. For the treatment of oral moniliasis in children in which diarrhoea was the commonest complication POHOWALLA, ARORA & SINGH (1957) reported 75 % clinical cure.

ANDLEIGH (1958a) reviewed the literature on *onchomycosis* and discussed the possibilities of the use of Mycostatin dermatological cream for treating *Monilia*. MITRA, DUTTA & MUKHERJEE (1959) have discussed the use of Nystatin in vaginal candidiasis based on the clinical valuation of the drug on 62 gynaecological and 5 obstetric cases. According to him Nystatin the new antifungal antibiotic might meet all the requirements for the treatment of vaginal candidiasis. POHOWALLA & KAUL (1959) found the oral administration of Nystatin to give satisfactory results in treatment of clinical and subclinical oral moniliasis in the newborn.

RAJAGOPALAN (1959) in his studies with Nystatin in the treatment of both cutaneous and mucous membrane lesions of *Candida albicans* found the drug to give good results with only external application of Nystatin against *Candida* infections. RAMAN & MARQUIS (1959) in their report on the isolation of *C. albicans* from *Candida* infection treated with broad spectrum antibiotics found these drugs to have an effect on the normal flora of alimentary canal. In the positive cases, however, none showed any symptoms of *C. albicans* infection. SATYAVATHI (1959a) treated 50 symptomatic cases of vaginal moniliasis with Mycostatin vaginal tablets and kept a record of the treated and untreated ones till delivery. The results indicated that 15 % of the untreated women transmitted the infection to the infants and none in the treated group.

The first nutritional study of *Candida albicans* is by SIRSI, RAO & RAMAKRISHNAN (1959) who in an attempt to study the mode of action of Nystatin in inhibiting the growth of *C. albicans* focussed their attention on the various aspects of the metabolism of the fungus. Since glucose is known to be a good carbon source for the growth of *C. albicans* and the derangement of carbohydrate metabolism as observed in diabetes favours *Candida* infection the effect of Nystatin on the utilisation of some of the intermediary products of the carbohydrate metabolism were investigated by them.

RHINOSPORIDIOSIS

Rhinosporidiosis is a fungus disease, endemic in India and Ceylon, caused by *Rhinosporidium Seeberi*. The infection usually occurs in the mucous membrane of nose, eyes, ears, larynx and occasionally the vagina, penis and skin characterised by the friable, sessile or pedunculated polyp.

The early history of the disease is available from the summarised accounts given by WRIGHT (1922b) and ALLEN & DAVE (1936). O'KINEALY in 1894, at the Medical College Calcutta, was the first to record the disease, in a muslim from Behar suffering from a growth in his left nostril; the record, however, was not published till 1903. The pathogen isolated by him was identified and named as *Rhinosporidium Kinealyi* by MINCHIN & FANTHAM (1905). INGRAM (1910) described one conjunctival polyp and one papilloma of the penis showing typical cysts of *R. Kinealyi* and claimed that till then no such case had been described except those in nose and nasopharynx. SEEBER (1912) found the organism *Rhinosporidium Kinealyi* to be identical with the organism described by him in 1900 and named it *Rhinosporidium Seeberi*. TRIMURTI (1914) gave a summary of the Indian cases of rhinosporidiosis. KIRKPATRIK (1916) recorded infection of lacrhymal sac from a patient in Madras. WRIGHT (1922) in Madras studied the rhinosporidiosis of conjunctiva of three Eurasian boys among 151 inmates of an Asylum (91 boys and 60 girls) and (1922a) recorded the successful treatment of *R. Kinealyi* infection by using tartarated antimony with a case note indicating the infection focus of the lachrymal sac. WRIGHT in the same year (1922b) gave a brief historical account of the disease with a detailed description of symptoms of three patients studied by him. He found certain interesting features of the disease and the focus of infection suggested the disease to be dust or water borne. All attempts at animal inoculation on monkeys gave negative results. WRIGHT & TRIMURTI (1922) discussed briefly the history of the disease, nature of local lesions and microscopic appearance of the pathogen. The disease was found to be common among inhabitants from West Coast in Madras, the majority of them being boys or young adults. All experiments on animal inoculation in monkeys proved unsuccessful. TRIMURTI (1924) described *Rhinosporidium Seeberi* (*Kinealyi* infection) for the first time in an Indian woman with a disease history for the past 20 years.

VASUDEVAN (1929) found a female with a growth on the fauces and a polyp in the pharynx. KURUP (1931) described rhinosporidiosis infection of 8 years duration of a muslim woman. The disease consisted of a tumor arising from the nasopharynx, the pathogen, however, was not isolated. He further described a rhinosporidiosis occurring as a growth on the eyelid of a twelve year old boy, the etiological agent being *Rhinosporidium Kinealyi*. In the succeeding year VASUDEVAN (1932) described a parasite resembling in some respects *Rhinosporidium Seeberi* from the tissue lesion of forearm of patient in Ceylon. MACKENZIE (1933) reported the disease in an Englishman who probably contracted the infection in Kirkee near Poona. NORONHA (1933) put on record the occurrence of rhinosporidiosis among sand workers in Poona and described the histology of the rhinosporidial polyp. His observations led him to believe that the incidence of the disease was causally related to the occupation of sand collection as almost all his patients were sandworkers and in absence of direct evidence the author considered the infection to be water borne. Same year NORONHA (1933a) published further evidence to show that liberation of spores by a mature rhinosporidial sporangium resulted in a localised inflamation with formation, at least in some instance, of miliary substances.

ALLEN & DAVE (1936) briefly reviewed the Indian work of rhinosporidiosis and presented the results of their study of sixty cases of the disease in the Central Provinces. The disease was found to be predominant in males particularly among young adults and was probably transmitted through dust or water. They found the diagnosis to be rather simple and proposed lines of treatment. A detailed account of the types of rhinosporidial tumors, their clinical features, sites of infection and various aspects of treatment have also been given.

ANDLEIGH (1951) reported two fungal infections, one of them being rhinosporidiosis of left nostril of a youngster of 14 years of age but was unable to grow the causal pathogen in culture. Again in 1952 he described three more cases of rhinosporidiosis. In all these a history of swimming was associated with the onset of diseases. PURANDRE & DEORAS (1953) reviewed 100 patients suffering from rhinosporidial infection among which only one was female. They found that in 91 patients the seat of lesion was nose and nasopharynx, in 5 patients the seat was ocular and that larynx, urethra and skin each accounted for one patient. Clinical features and certain aspects of infection are discussed and compared with the results of other investigations. NAVANI & TYAGI (1957) reported a rhinosporidiosis from western Uttar Pradesh based on clinical diagnosis, gross findings and microscopic examinations although fungus was not isolated in culture. LAHIRI (1958) reported from Bengal three persons including a female suffering from this disease the pathogen isolated being *Rhinosporidium Seeberi*.

BLASTOMYCOSIS

Blastomycosis is a chronic suppurative nature of fungus infection caused by *Blastomyces dermatitidis* which can attack any part of the body but commonly the skin and internal organs such as bones and lungs. It is of common occurrence in North and South America but infrequent in India there being only five scattered reports from different places so far.

Blastomycosis was first reported from India by GANGULI (1925) from the coolies in the gardens of Duars, confirmed by positive microscopic evidence. G. PANJA in the same year (1925) reported a case of generalised blastomycosis with detailed description of the symptoms. The pustules when examined in KOH sol. had revealed typical fungus. He also suggested certain lines of treatment.

DeMello & Rodrigues (1929) studied blastomycosis infection of nails in a youth of 18 years from Portugese India caused by *Monilia*.

Andleigh (1951) came across a primary cutaneous blastomycosis as determined by clinical history and laboratory findings. He described in detail the disease and the causal organism and also gave the method of differentiating the disease from coccidioidomycosis. Jayaram, Sirisi, Ahmad & Dayalu (1952) reported in detail the blastomycosis of a lung of a male patient of sixty years. Cultural examination showed budding yeast-like spherical cells with double contoured thick refractile wall. Mukherjee in 1953 described a North American blastomycosis, both cutaneous and systemic in nature. The patient died after an illness of 12 weeks.

SPOROTRICHOSIS

Sporotrichosis is a disease, mainly of the skin caused by the species of *Sporotrichum*. It usually develops in the form of subacute or granulomatous lesion of the skin, underlying subcutaneous tissues and lymph nodes. The disease is scarce in India and altogether four cases are reported so far.

L. M. Ghosh (1932)for the first time recorded sporotrichosis. Later Panja, Dey & Ghosh (1947) recorded sporotrichosis of the skin of an Indian vegetable seller caused by a new species *Sporotrichum tropicale* and claimed it to be the second authentic case of *sporotrichosis* reported from India. The isolated species was reported to be pathogenic to common laboratory animals. Its growth showed resemblance to *Sporotrichum schencki-beurmannii*, the European type species in colour, but differed from it in its biochemical reactions, morphology and some other features. It also differed from other species of *Sporotrichum* in many respects, and was named *Sporotrichum* (*Rhinocladium*) *tropicale* sp. nov.

Dey, Saikia & Majumdar (1958) recorded two cases of sporotrichosis in Assam. The fungus isolated from one on inoculation in white rats produced lesions in the testes. The direct microscopic examination of the scrapings of lesions revealed the presence of yeast-like bodies of a fungus. The patients responded to the treatment with potassium iodide.

CHROMOBLASTOMYCOSIS

Chromoblastomycosis is a chronic granulomatous, sometimes ulcerative, cutaneous, mycotic infection affecting mostly the extremities and the head.

Andleigh in 1953 reported one probable case of chromoblastomycosis based on the clinical diagnosis only along with a detailed discussion on the symptoms, pathology and diagnosis of the disease in general, but Kakoti & Dey in (1957) recording a case of chromoblastomycosis of the external female genitalia claimed it as the first report from India. The diagnosis was confirmed by direct, histopathological and cultural tests by finding typical spores in the tissue and the typical growth in culture of the pathogen *Hormodendron compactum*. Metastasis which is rare was noted in original lymph nodes (*inguinal lymphnodes*). THOMAS, JOB & HADLEY (1957) reported the first case of chromoblastomycosis in continental Asia in a 35 years old man with verrucous lesions on the right foot over a period of 12 years. The clinical, histological and cultural features closely resemble those of the previously reported cases. The pathogen *Hormodendron compactum* was isolated.

RAJAN, KANDHARI & THIRUMALACHAR (1958) reported from Poona an unusual clinical type of chromoblastomycosis in a boy of 7 years with prolonged severe illness and ultimate death. The mycological studies revealed an unusual chromogenic fungus, unlike the usual fungus causing the disease. The present fungus has both yeast and mycelial phases in the diseased tissue and the culture.

GOKHALE, THIRUMALACHAR & PADHYE (1959) described an interesting case of black papulosquamous skin caused by *Hormodendron* sp. which was repeatedly isolated froming a compact olive-black growth on most of the mediums. The isolation was made from a case which did not show symptoms of chromoblastomycosis but resembled more of *tinea nigra* type of infection.

HISTOPLASMOSIS

Histoplasmosis is a mycotic infection primarily involving the reticul-endothelial system in various tissues and organs, of acute, sub-acute or chronic, localized or disseminated nature. Only three cases are reported.

PANJA & SEN (1954) described for the first time the disease histoplasmosis. The skin as well as the viscera were involved, typical intracellular parasites were revealed both in microscopic examination and biopsy. Treatment with various drugs were of no avail.

SEN GUPTA, RAO, BANERJEE, CHAKRABORTY & RAY (1957) reported progressive histoplasmosis by clinical features, pathological and mycological examinations. KALRA, BORCAR & REBELLO (1957) successfully isolated two strains of *Histoplasma* species probably *H. duboisii* from Poona. One strain was isolated from soil and the other from the sputum with positive roentgenological test for infection of lungs.

COCCIDIOIDOMYCOSIS

Coccidioidomycosis is chiefly a respiratory disease of endemic nature. So far there is only one probable report from India. Das, Chatterjee & Deb Sarkar (1958) reported coccidioidomycosis with clinical picture and other characters suggestive of the disease, along with a short discussion on coccidioidomycosis in general. Although they were able to demonstrate bodies similar to spherules of *C. immitis* in wet preparation of the discharge and histological section, in the absence of the coccidioidin test the identity of the disease remains doubtful.

BIBLIOGRAPHY

1. Introduction and Maduromycosis

Agostino Bassi. 1835. Del mal del segno calcinaccio o muscardino malattia che affligge i bachi de seta e sul modo de liberarne le gigattaie anche le piu infestate. Parte I. Teorica, pp. I—IX, 1—67, presso Orcesi, Lodi. 1835.

Ahmad, M. A., 1929. Two cases of surgical interest. Indian Med. Gaz. **64:** 85—86.

Andleigh, H. S., 1951. Laboratory diagnosis of mycotic infection. J. Indian Med. Ass. **20:** 368—371.

Andleigh, H. S. 1953. Chromoblastomycosis — review with a favourable case. Indian J. Med. Sci. **7:** 409—414.

Andleigh, H. S. 1957. Etiology of maduromycosis in India. Mycopath. et Mycol. Appl. **8:** 138—153.

Andleigh, H. S. 1957a. In vitro study of antifungal activity of Pentamidine and Stilbamidine. *ibid.* **8:** 135—137.

Andleigh, H. S. 1958. Investigations into the role of fungi in pulmonary diseases in India. Amer. Rev. Tuberculosis. **78:** 644—646.

Ainsworth, G. C., 1951. Medical Mycology. An Introduction to its problems. Pitman & Sons Ltd. London.

Ainsworth, G. C., 1951a. A century of medical and veterinary mycology in Britain. Trans. Brit. mycol. Soc. **34:** 1—16.

Allen, F. R. K. W. & M. L. Dave. 1936. The treatment of Rhinosporidiosis in man based on the study of sixty cases. Indian med. Gaz. **71:** 376—394.

Basu, S. P., A. K. Banerjee & A. Rao. 1959. Mycetoma — a radiological study. Symposium of fungus diseases in India.

Berkeley, M. J., 1846. Observations, botanical and physiological on the potato murrain. J. R. hort. Soc. London **1:** 9—34.

Berkeley, M. J., 1863. Observations on a peculiar mode of fructification in *Chionyphe Cartery*. Berk. J. linn. Soc. **8:** 139—142. P. 10.

Berkeley, M. J., 1863a. The fungus foot of India. Intellectual observer. **2:** 248—257

Blackley, C. H., 1873. Experimental researches on the causes and nature of *Catarrhus aestivus*. (hay fever or hay asthma) London.

Boccaro, J. E., 1893. An analysis of one hundred cases of mycetoma. Lancet **2:** 797—798.

Boccaro, J. E., 1894. Clinical notes on mycetoma differentiating the pale from the black variety. Indian med. Gaz. **29:** 451.

Boyce, R. & N. F. Surveyor., 1894. Upon the existence of more than one fungus in Madura disease (mycetoma). Philos. Trans. London. **185:** 1—14.

Bradfield, E. W. C. & A. Vasudevan, 1927. Mycetoma. *ibid.* **62:** 633—634.

Bringal, 1859. cited by H. V. Carter. Trans. med. and phys. Soc. Bombay. 1860. **6:** 104—142.

Bristowe, 1870. Cited in Manual of Tropical Medicine by A. Castellani & A. J. Chalmers, 1919.

Burill, T. J., 1878. Pear blight. Trans. Ill. State hort. Soc. **11:** 114—116.

Burill, T. J., 1881. Anthrax of fruit trees. Proc. Amer. Ass. Adv. Sci. **29:** 583—597.

Carter, H. V., 1860. On a new and striking form of fungus disease, principally affecting the foot and prevailing endemically in many parts of India. Trans. med. and phys. Soc. Bombay, **6:** 104—142.

CARTER, H. V., 1861. On mycetoma or the fungus disease of India including notes of recent cases and new observations on the structure and of the entophytic growth. *ibid.* **7:** 206—221.

CARTER, H. V., 1862. Notes on mycetoma, the fungus disease of India. *ibid.* **8:** Appendix 26.

CARTER, H. V., 1874. On mycetoma or the fungus disease of India. London. 118 pp. 11 pls.

CARTER, H. V., 1874a. On the nature of mycetoma or the fungus disease in India. Lancet. London. **2:** 44—46; 113—115.

CARTER, H. V., 1886. Cited by GAMMEL, J. A., M. MISKDJIAN & H. S. THATCHER, Arch. Derm. and Syph. **13:** 66—77.

CHAKRABORTY, A. N., S. GHOSH & A. K. BANERJEE, 1953. Review on the study of skin disease in animal communicable to man. Indian med. Gaz. **88:** 152—153.

CHAUDHURI, R. N., 1957. Fungus diseases in man. Bull. Cal. Sch. Trop. Med. **5:** 73—75.

COLEBROOK, L., 1848. Cited by H. V. CARTER. Trans. med. and phys. Soc. Bombay. 1860. **6:** 104—142.

COLLAS, 1861. Leçon sur la dégéneration endémique des os du pied. Pondicherry.

COLLAS, 1883. Arch. Med. Nav. **81:** 204.

CUNNINGHAM, D. D., 1873. Microscopic examination of air. pp. 1—58. Calcutta.

DE BARY, A., 1853. Die Brandpilze. pp. 1—144. Berlin.

DEY, N. C. 1953. A review of ringworm of hair in India. Indian med. Gaz. **88:** 194—196.

DEY, N. C., 1959. Medical Mycology. Pressagents, Calcutta.

DEY, N. C., 1959a. Granulomas of the skin caused by fungi in India. Symposium of fungus diseases in India.

DODGE, C. W., 1935 Medical Mycology. Fungus diseases of man and other mammals. C. V. Mosby Company.

DYMOCK, D. F., 1881. Fungus disease — recurrence after amputation. Indian med. Gaz. **16:** 56.

EYRE, E. W., 1860. Account of a peculiar disease (tubercular) of the foot. Indian Ann. med. Sci. **12:** 513—520.

FITZERALD, H. G., 1932. A mycetoma-like condition of the foot. Indian med. Gaz. **67:** 517—518.

GAMMEL, J. A., H. MISKDJIAN & H. S. THATCHER. Madurafoot (mycetoma) the blackgrain variety in a native American. Arch. Derm. and Syph. **13:** 66—77.

GHOSH, L. M., N. C. DEY & D. PANJA, 1950. Madurafoot (mycetoma). Indian med. Gaz. **85:** 288.

GILL, 1842. Cited by H. V. CARTER. Trans. med. and phys. Soc. Bombay, 1860. **6:** 104—142.

GODFREY, J., 1844. Cited by H. V. CARTER. Trans. med. and phys. Soc. Bombay 1860. **6:** 104—142.

GODFREY, J., 1846. Disease of the foot not hitherto described. Lancet. **1:** 593—594.

HOGG, 1872. Cited in Manual of Tropical Medicine by A. CASTELLANI and A. J. CHALMERS, 1919.

HOLMSTED, 1870. Cited in Manual of Tropical Medicine by A. CASTELLANI and A. J. CHALMERS, 1919.

KAKOTI, L. M. & N. C. DEY, 1956. Mycetoma caused by *Glenospora Semoni.* Indian J. med. Sci. **10:** 889—891.

KANTHACK, A. A., 1893. Madura disease (mycetoma) and actinomycosis. J. Path. and Bact. **1:** 140—162.

LAGENBECK, 1839. Bacterial and mycotic infections of Man. Edited by RENE J. DUBOIS. C. V. Mosby Company, 1948.

MAITLAND, J., 1898. Case of mycetoma of the abdominal wall. Indian med. Gaz. **33:** 97.

PALMER, F. J., 1926. A case of madurafoot treated by chemotherapy. Indian med. Gaz. **61:** 74.

PALMER, F. J., 1928. A second case of madurafoot treated by chemotherapy with apparent cure. *ibid.* **63:** 530—531.

PANJA, G. & S. N. CHAUDHURI, 1958. Mycetoma of the foot-melanoid variety. Indian J. Dermat. **3:** 24—26.

PRASAD, K, 1906. Notes on a case of fungus disease in India — mycetoma or madurafoot. Indian med. Gaz. **41:** 139—140.
RAO, A., 1959. Fungus disease in India — a historical review. Symposium of fungus diseases in India.
RAO, M. G. R., 1928. A case of mycetoma of the hand and foot. Indian med. Gaz. **63:** 329.
RAY, P. N. & B. P. TRIBEDI, 1938 — 39. Primary actinomycosis of the breast. Brit. J. Surg. **26:** 30—34.
RUSTOMJI, B., 1860. Case of diaeased foot. Trans. med. and phys. Soc. Bombay. **5:** 230—241.
SCHOENLEIN, 1839. Cited in Bacterial and mycotic infections of man. Edited by RENE J. DUBOIS. C.V. Mosby Company 1948.
SMITH, J., 1898. Notes on a case of Mycetoma of the neck. Indian med. Gaz. **35:** 96.
TRIBEDI, B. P. & B. N. MUKERJEE, 1939. Actinomycosis and mycotic lesions with special reference to madurafoot. Brit. J. Surg. **27:** 256—265.
VASUDEVAN, A. & N. SESHADRINATHAN, 1929. A few observations on mycetoma — A preliminary communication. Indian J. med. Res. **17:** 170—173.
VINCENT, M. H., 1894. Cited by G. C. AINSWORTH. Trans. Brit. mycol. Soc. 1951. **34:** 1—16.

2. Dermatomycoses

ACTON, H. W., 1919. The diagnosis of acute infection of throat amongst troops of Mesopotamium expeditionary force. Indian J. med. Res. **6:** 152.
ACTON, H. W., 1919a. Mycotic affections of throat due to *Endomyces tropicalis*. *ibid.* **6:** 591.
ACTON, H. W. & N. C. DEY, 1934. A simple method of recovering typical cultures of dermatophytes from pleomorphic growth. Indian med. Gaz. **69:** 601.
ACTON, H. W. & L. M. GHOSH, 1934. *Tinea imbricata* (Tokelau) in Bengal. Indian med. Gaz. **69:** 426—430.
ACTON, H. W., & C. McGUIRE, 1927. *Tinea cruris* its manifestation, diagnosis and treatment. *ibid.* **62:** 24.
ACTON, H. W. & C. McGUIRE, 1929. A purulent folliculitis due to *Trichophyton violaceum* var. *indicum*. *ibid.* **64:** 24.
ACTON, H. W. & G. PANJA, 1927. Seborrhic dermatitis or Pityriasis capitis. Indian med. Gaz. **62:** 603.
ANDLEIGH, H. S., 1951. Mycotic infections. Indian J. med. Sci. **5:** 168—171.
ANDLEIGH, H. S., 1955. Comparison of different staining techniques with alkali clearing method used in the diagnosis of superficial fungus infection. *ibid.* **9:** 235—238.
ANDLEIGH, H. S., 1958. A note on the growth of *Malassezia furfur*. Mycopath. et Mycol. Appl. **9:** 20—22.
BANERJEE, B. N., 1957. Common skin diseases of hands in the tropics. Indian J. Dermat. **2:** 99—104.
BANERJEE, A. K., A. N. CHAKRABORTY, N. ROY & A. RAO, 1956. Localisation of alkali phosphate in certain skin diseases. A preliminary note. Bull. Cal. Sch. trop. Med. **4:** 125.
BANERJEE, A. K., H. N. RAY, T. N. GHOSH & A. N. CHAKRABORTY, 1957. Nuclear reaction in *Trichophyton rubrum*. *ibid.* **5:** 12.
BANERJEE, A. K., A. RAO & A. N. CHAKRABORTY, 1958. Observation on the incidence of dermatophyte infection. *ibid.* **6:** 9.
BANERJEE, A. K., A. RAO & A. N. CHAKRABORTY, 1959. Dermatomycosis — a clinical, mycological and therapeutic study. Symposium of fungus diseases in India.
BANERJEE, N., S. K. MAJUMDAR & S. K. BOSE, 1959. Chemotherapeutic investigations on Mycobacillin. Preliminary observations. ibid.
BARAT, D. B., 1955. Skin diseases in rural area amongst children. Indian J. med. Res. **43:** 178—179.

Behl, P. N. & M. D. Sharma, 1958. Incidence of mycotic infection in Delhi. Indian J. Dermat. **3:** 1—4.

Behl, P. N. & B. D. Kumar, 1958. Bradex-vioform in the treatment of fungus and other diseases. Indian J. med. Sci. **12:** 43—45.

Bhattacharya, H. C., H. N. Ray & A. N. Chakraborty, 1958. Alkaline phosphatase activity in *T. rubrum*. Bull. Cal. Sch. trop. med. **6:** 10.

Bhattacharya, H. e., H. N. Ray & A. N. Chakraborty, 1958a. Cytochemical demonstration of iron, calcium and lipoid in *T. rubrum*. *ibid*. **6:** 64.

Bhattacharya, K. C., H. N. Ray & A. K. Banerjee, 1959. Demonstration of lipase and succinic dehydrogenase activity in some dermatophytes and affection of skin. *ibid*. **7:** 9—11.

Castellani, A, 1905. Observations on *Dhobies itch* and other tropical trichophytic diseases. Brit. med. J. **2:** 1277—1279.

Castellani, A., 1910. Observations on a new species of *Tinea epidermophyton* found in *cruris*. Brit. J. Derm. **22:** 147—150.

Castellani, A., 1920. Milroy lectures on higher fungi in relation to human pathology J. trop. Med. **23:** 101.

Chakraborty, A. N. & A. K. Banerjee, 1956. Isoniazid and Paramino-salicylic acid in cutaneous nocardiosis. Bull. Cal. Sch. trop. Med. **4:** 130.

Chakraborty, A. N., S. Ghosh & A. K. Banerjee, 1953. Review on the study of the skin diseases in animals communicable to man. Indian med. Gaz. 88: 152—153.

Chakraborty, A. N., S. Ghosh & A. K. Banerjee, 1954. Animal skin disease communicable to man. Bull. Cal. Sch. trop. Med. **3:** 24—26.

Chakraborty, A. N., A. K. Banerjee & S. Ghosh, 1953. An unusual case of ringworm — a case report. Indian med. Gaz. 88: 268—169.

Chuckerbutty, B, 1958. Clinical trial of a new antimycotic drug (Multifungin). Indian J. Dermat. **3:** 118—119.

Das-Gupta, S. N. & S. K. Shome, 1959. Studies in Medical Mycology I. On the occurrence of mycotic diseases in Lucknow. Mycopath. et. Mycol. Appl. **10:** 177—186.

Das-Gupta, S. N. & S. K. Shome, 1959a. Nutritional properties of common Indian dermatophytes. Proc. Indian Sci. Congr. 1960.

DeMello, F., 1917—1918. *Trichophyton viannae* n.sp. The infecting agent in case of dermatomycosis. Indian J. med. Res. **5:** 222.

Desai, S., L. Marquis & M. L. Bhatt, 1959. Mycological study on common superficial mycoses. Symposium of fungus diseases in India.

Devi, K. S. & B. N. Dhanvantari, 1959. Fungal diseases of man. *ibid*.

Dey, N. C., 1941. Notes on common skin diseases III. Ringworm of scalp (Favus). Indian med. Gaz. **76:** 416.

Dey, N. C., 1953. A review of ringworm of hair in India. *ibid*. **88:** 194—96.

Dey, N. C., 1953a. *Trichophyton purpureum* infection in India. *ibid*. **88:** 525—526.

Dey, N. C., 1957. Superficial *pustular folliculitis* including *sycosis barbae* in India. Indian J. Dermat. **2:** 61—64.

Dey, N. C. 1959. Nomenclature of pathogenic fungi (names recommended to be used in India). *ibid*. **4:** 88—91.

Dey, N. C., 1959a. A natural classification of mycotic diseases. Symposium of fungus diseases in India.

Dey, N. C., 1959b. Ringworm of scalp in children. *ibid*.

Dey, N. C. & P. A. Maplestone, 1935. Ringworm of scalp in India. Indian med. Gaz. **70:** 541—544.

Dey, N. C. & P. A. Maplestone, 1936. Favus in India. Indian J. med. Res. **23:** 687.

Dey, N. C. & P. A. Maplestone, 1941, *Trichophyton crateriforme* in India (*Tinea tonsurans*) black dot ringworm. Indian med. Gaz. **76:** 410.

Dey, N. C. & P. A. Maplestone, 1942, *Tinea imbricata* in India. ibid. **77:** 56.

Dey, N. C. & L. M. Ghosh, 1944. Studies on ringworm. I. *Microsporon Audouini* infection in India. *ibid*. **79:** 51.

Dey, N. C. & L. M. Ghosh, 1945. Studies on ringworm. II. Therapy of *Tinea capitis* by gonadotropic hormones. *ibid*. **80:** 381.

DEY, N. C. & L. M. KAKOTI, 1955. *Microsporum gypseum* in India. J. Indian med. Ass. **25:** 160—164.

DEY, N. C. & L. M. KAKOTI, 1956. *Faviformectothrix Trichophytosis* in India. Indian J. Dermat. **1:** 105—107.

DUTTA, D. P., S. K. MAJUMDAR & S. K. BOSE, 1955. Studies in antifungal antibiotics. Action against skin pathogen. Sci. and Cult. **20:** 449.

GHOSH, L. M., 1940. Notes on common skin diseases. I Ringworm of the foot. Indian med. Gaz. **75:** 95.

GHOSH, L. M., 1941. Notes on common skin diseases. II Ringworm of scalp. *ibid.* **76:** 268.

GHOSH, L. M., 1948. An analysis of 50,000 cases as seen in the out patient department of the Tropical School of Medicine, Calcutta during the 5 years from 1942—1946. *ibid.* **83:** 493—500.

GHOSH, L. M., & P. A. MAPLESTONE, 1940. Epidermophytosis in a very young child. *ibid.* **75:** 95.

GHOSH, L. M. & P. A. MAPLESTONE, 1941. An improved method of growing pure cultures of ringworm fungi. Indian J. med. Res. **29:** 691—692.

GHOSH, L. M. & D. PANJA, 1946. Studies on ringworm. III. Prophylactic measures. Indian med. Gaz. **81:** 73—75.

GHOSH, L. M. & B. K. SEN, 1947. A case of dermatomyositis. Indian med. Gaz. **82:** 208.

GHOSH, L. M., N. C. DEY & D. PANJA, 1947. Studies on ringworm. IV. Incidence of *Trichophyton gypseum* in India. *ibid.* **82:** 73—75.

GHOSH, L. M., D. PANJA & N. C. DEY, 1948. Studies on ringworm. V. Fungus diseases of the nails and the surrounding tissue. *ibid.* **83**: 215—16.

GHOSH, L. M., D. PANJA & N. C. DEY, 1948a. Studies of ringworm. VI. Ringworm of the glabrous skin. *ibid.* **83:** 272—74.

GOKHALE, B. B., M. J. THIRUMALACHAR & A. A. PADHYE, 1959. Observation on mycosis in patients examined at the Sassoon Hospital. Poona. Symposium of Fungus diseases in India.

GOVINDAN NAIR, V. 1942. Ringworm in Vizagapatam. J. Indian med. Ass. **11:** 368.

GUPTA, N. & N. BASU. 1958. Clinical evaluation of Bradex-vioform cream in fungal infection including *Psoriasis*. Indian J. Dermat. **3:** 70—71.

GUPTA, R. N. & S. K. SHOME, 1959. Dermatomycosis in Uttar Pradesh — An analysis of 620 cases. J. Indian med. Ass. **33:** 39—42.

LAHIRI, K. D., 1958. Favus. Indian J. Dermat. **3:** 35—36.

LAHIRI, K. D., 1958a. *Tinea pedis*. *ibid.* **3:** 50—56.

LAHIRI, K. D., S. SEN, R. KUNDU & M. R. LAHIRI, 1958. *Tinea versicolor*. *ibid.* **3:** 57—58.

MACKIE, F. P. 1921—22. Visceral infection due to higher fungi. Indian J. med. Res. **9:** 781.

MAJUMDAR, S. K. & S. K. BOSE, 1955. Studies on antifungal antibiotics. I. Antifungal micro-organisms in Indian fruits and vegetables. J. Sci. Ind. Res. **14c:** 126—128

MAJUMDAR, S. K. & S. K. BOSE, 1955a. Studies on antifungal antibiotics. II. Distribution of antifungal micro-organisms in soils. *ibid.* **14c:** 165—167.

MAJUMDAR, T. D., 1958. Dermatophytid or 'ID' reactions. Indian J. Dermat. **3:** 59—62.

MAJUMDAR, T. D., B. SEN GUPTA, H. N. DAS GUPTA & B. CHAKRAVARTY, 1956. Experience with crotonyl-n-ethyl-o-toluedine cream as an aid to local treatment to pruritic skin disease. J. Indian med. Ass. **26:** 132—134.

MAPLESTONE, P. A. & N. C. DEY, 1938. Laboratory tests on fungistatic and fungicidal effects of various substances. Indian J. med. Res. **25:** 603.

MAPLESTONE, P. A. & N. C. DEY, 1939. A *microsporon* new to India. Indian med. Gaz. **74:** 148—151.

MAPLESTONE, P. A. & N. C. DEY, 1941. Further laboratory tests on fungistatic and fungicidal effects of various substances. Indian J. med. Res. **29:** 23.

MITRA, A. K. 1940. *Otomycosis* in a woman caused by *Aspergillus niger* Tieghem. Curr. Sci. **9:** 371.

MITRA, S. C., 1958. *Kerion* with three case notes. Indian J. Dermat. **3:** 33—34.

MITRA, S. C. & B. N. BANERJEE. 1958. Further work on evaluation on the new antimycotic drug (Bradex-vioform). Indian J. Dermat. **3**: 65—69.

MUKERJEE, A. K., S. N. CHAUDHURI & H. N. RAY, 1958. A new method of staining parasitic fungi in skin and nail lesions. Bull. Cal. Sch. trop. med. **6**: 10.

MUKERJEE, A. K., S. N. CHAUDHURI & H. N. RAY, 1958a. Hotchkiss and McManus stain for demonstration of ringworm fungus in scrapings from skin and nails. *ibid*,. **6**: 65—66.

PANJA, D., 1958. 'Ceiro and Podopompholyx'. Indian J. Dermat. **3**: 58.

PANJA, G., 1925. A case of melanoderma. Indian med. Gaz. **60**: 476—477.

PANJA, G., 1956. Trichophytide, J. Indian med. Ass. **27**: 401.

PANJA, G., 1958. *Tinea cruris* and *Tinea circinata.* Indian J. Dermat. **3**: 44—49.

PANJA, G., 1959. Cultivation of the *Malassezia* (*M. furfur* and *P. ovale*), their successful isolation, morphology pathology and classification. Symposium of fungus diseases in India.

PANJA, G., 1959a. Seborrohoeic dermatitis. *ibid.*

PANJA, G., 1959b. Study of ringworm infection on glabrous skin in Calcutta. *ibid.*

PANJA, G. 1959c. Therapeutic problem of the dermatomycotic infection in India. *ibid.*

PANJA, R. K., 1958. *Tinea barbae*, Indian J. Dermat. **3**: 36—37.

POWELL, A., 1900. Ringworm in Assam. Indian med. Gaz. **35**: 109.

RAY, S. N., 1958. Treatment of ringworm of scalp. (*Tinea tonsurans*). Indian J. Dermat. **3**: 63—64.

SHOME, S. K., 1959. *Trichophyton verrucosum* — the etiological agent for ringworm of glabrous skin — a new report. Ph. D. Thesis Lucknow University.

SHOME, S. K., 1959a. Ringworm of scalp in Uttar Pradesh. *ibid.* also Proc. Indian Sci. Congr. 1960.

SHOME, S. K., 1959b. Pigmentation in *Trichophyton rubrum.* Ph. D. Thesis. Lucknow University.

SHOME, S. K., 1959c. Dual phenomenon and the dermatophytes. *ibid.*

SHOME, S. K., 1959d. Antibiosis between *Bacillus subtilis* and dermatophytes. *ibid.*

SHOME, S. K., 1959e. Preliminary studies on the enzymes of dermatophytes *ibid.*

SHOME, S. K., 1959f. Colorimetric estimation of dehydrogenase activity in *T. rubrum* and *T. mentagrophytes. ibid.*

SHOME, S. K., 1959g. Phosphorylase activity in *T. rubrum* and *T. mentagrophytes ibid.*

SHOME, S. K. & J. P. VERMA, 1959. Deamination of certain amino acids by *T. rubrum* and *T. mentagrophytes. ibid.*

SRIVASTAVA, O. P., 1957. *Trichosporon cutaneum* from the foot Skin of man and the effect of antifungal drugs on it. J. Indian bot. Soc. **36**: 268—271.

SRIVASTAVA, O. P., 1959. Antifungal activity of some sulphur containing compounds against dermatophytes and yeast-like fungi. J. Sci. Ind. Res. **18c**: 25.

SRIVASTAVA, O. P. & R. N. GUPTA, 1958. Survey of human skin fungi in Lucknow and the effect of diarylsulphones, sulphoxides and sulphides on *Trichophyton rubrum.* J. Sci. Ind. Res. **17c**: 87—90.

VERMA, B, 1959. Tinea versicolor — A study of 125 cases in Baroda (India). Symposium of fungus diseases in India.

WADE BROWN, 1898. Dhobies itch. Correspondence to the Editor, Indian med. Gaz. **33**: 357.

3. Actinomycosis

ACTON, H. W. & C. MCGUIRE, 1930. Keratolysis plantare sulcatum, a lesion due to an actinomycotic fungus. Indian med. Gaz. **66**: 65.

ACTON, H. W. & C. MCGUIRE, 1931. Actinomycotic lesions of the skin of the hands and feet due to *Actinomyces Keralolytica. ibid.* **67**: 65.

ANDLEIGH, H. S., 1951. Two rare cases of fungus infection of skin in Rajasthan, actinomycosis and rhinosporidiosis. *ibid.* **86**: 100.

ANDLEIGH, H. S., 1955. Actinomycosis with special reference to streptomycin sensitivity. J. Indian med. Ass. **24**: 597—598.

BANERJEE, B. N., 1958. Primary actinomycosis of the skin in tropics, Indian J. Dermat. **3:** 8—13.
BASU, C., 1937. Notes on a new strain of *actinomyces* obtained from blood culture. Indian J. med. Res. **25:** 325—27.
BASU, C. C., 1952. Human actinomycosis. Indian med. Gaz. **87:** 237—244.
PANJA, G., 1948. An unusual case of actinomycosis of cheek. *ibid.* **83:** 416.
PANJA, G., 1956. Two interesting cases of actinomycosis. Indian J. Dermat. **1:** 137—138.
RANAVRE, M. N., 1951. *Actimomyces* of the lung. Indian J. med. Sci. **5:** 63—65.
SAHA, B., 1918. A case of actinomycotic pleuromediastinitis. Indian med. Gaz. **53:** 11.
SEN, S. N., 1951. Pulmonary actinomycosis. *ibid.* **86:** 100.
SUBRAMANIAM, R., 1952. Actinomycosis of the lung. J. Indian med. Ass. **21:** 367.
SUR, T. 1918. Observations on three cases of *actinomycosis hominis*. Indian med. Gaz. **53:** 5.

4. Candidiasis

ANDLEIGH, H. S., 1958. Onchymycosis. Indian J. Dermat. **3:** 40—43.
BANERJEE, N. B., 1958. Moniliasis with a case note of an unusual and interesting case. *ibid.* **3:** 29—32.
BANERJEE, D. & I. M. GUPTA, 1956. Role of *Candida albicans* in an acute respiratory infection. J. Indian med. Ass. **27:** 58—61.
BATT, J. V. & M. V. SHETTY, 1946. An instance of the occurrence of *Monilia albicans* (*Candida albicans*) in dental caries. Curr. Sci. **15:** 228.
BASU, N., R. MAJUMDAR, A. RAO & A. K. BANERJEE, 1959. *Candida* in the faeces and its relation with similar organism in the oral cavity and the hand. Symposium of fungus diseases in India.
CHAKRABORTY, S. C., H. S. RANDHWA, B. ROY & R. VISHWANATHAN, 1959. *Candida* species in throat swab culture of normal population and also in sputum culture of bronchopulmonary diseases in India. *ibid.*
DAFTARY, V., 1959. Vaginal discharges. *ibid.*
DESAI, S. A., L. MARQUIS & M. L. BHATT, 1959. Investigation on a case of vulvovaginitis in children. *ibid.*
DEY, N. C., 1959. Pulmonary moniliasis. *ibid.*
KASLIWAL, R. M. & H. S. ANDLEIGH, 1958. An interesting case of bronchopulmonary moniliasis. Indian J. med. Sci. **8:** 95—97.
MAPLESTONE, P. A. & N. C. DEY, 1939. The use of dyes in various fungal infections. Indian med. Gaz. **74:** 391.
MITRA, S., R. DUTTA & T. MUKHERJEE, 1959. Nystatin in vaginal candidiasis. Symposium of Fungus diseases in India.
PANJA, D. & A. K. BANERJEE, 1950. *Perionychia* due to monilial infection. Indian med. Gaz. **85:** 137—138.
POHOWALLA, J. N., M. M. ARORA & B. N. SINGH, 1957. Nystatin in treatment of oral moniliasis in children. Indian J. med. Sci. **11:** 15—18.
POHOWALLA, J. N. & K. K. KAUL, 1959. Oral moniliasis in new borns in relation to vaginal moniliasis in mothers. Symposium of fungus diseases in India.
PURANDARE, B. N. & M. R. NARVEKAR, 1959. Fungus infection in non pregnant women complaining of Leucorrhoea. *ibid.*
RAJAGOPALAN, S., 1959. Nystatin in the treatment of *Candida albicans* infection. *ibid.*
RAMAN, K. & L. MARQUIS, 1959. Isolation of *C. albicans* in cases treated with broad spectrum antibiotics. A preliminary report. *ibid.*
SATYAVATI, C., 1959. Incidence of *vaginal moniliasis* in antenatal clinic of K. G. Hospital, Visakhapatnam. *ibid.*
SHELMIRE, B., 1925. Thrush infection of the skin. Arch. Derm. Syphilol. **12:** 789—813.
SHOME, S. K., 1959, Studies in Medical Mycology II. Candidiasis of Scalp. Mycopath. et Mycol. Appl. **10:** 187—190.
SINGH, K., 1950. Pulmonary moniliasis. Indian med. Gaz. **85:** 10—14.

Sirsi, M., G. Ramananda Rao & T. Ramakrishnan, 1959. Studies on the mode of action of Nystatin. Symposium of Fungus diseases in India.

Srivastava, O. P., B. M. Gupta & B. Mukherjee, 1958. Morphology and physiology of four strains of yeasts from human faeces and their in vitro sensitivity to Nystatin. J. Indian bot. Soc. **37**: 65—69.

5. Rhinosporidiosis

Allen, F. R. W. K. & M. L. Dave, 1936. The treatment of rhinosporidiosis in man based on the study of sixty cases. Indian Med. Gaz. **71**: 376—394.

Andleigh, H. S., 1951. Two rare cases of fungus infection of the skin in Rajasthan. Actinomycosis and rhinosporidiosis. *ibid.* **86**: 100.

Andleigh, H. S., 1952. Rhinosporidiosis in Rajasthan. Indian med. Sci. **6**: 16——22

Ingram, A. C., 1910. *Rhinosporidium Kinealyi* in unusual situations. Lancet. **2**: 726.

Kurup, P. K., 1931. *Rhinosporidium Kinealyi* infection. Indian med. Gaz. **66**: 239.

Kirpatrick, H., 1916. *Rhinosporidium* of the lachrymal sac. Opthalmoscope. **14**: 477.

Lahiri, K. D., 1958. Rhinosporidiosis. Indian J. Dermat. **3**: 27—28.

Mackenzie, K. W., 1933. *Rhinosporidium Seeberi.* J. Laryng. and Otol. **48**: 107.

Minchin, E. A. & H. B. Fantham, 1905. *Rhinosporidium Kinealyi.* n.g., n. sp. A new sporozoon from the mucous membrane of the septum nasi of man. Quart. J. micr. Sci. **49**: 521.

Navani, H. & G. K. Tyagi, 1957. Rhinosporidiosis in western Uttar Pradesh. J. Indian med. Ass. **28**: 474—475.

Noronha, A. J., 1933. A preliminary note on the prevalence of rhinosporidiosis among sand workers in Poona with a brief description of some histological features of some rhinosporidial polypus. J. trop. med. Hyg. **36**: 115—120.

Noronha, A. J., 1933a. Further observation on rhinosporidiosis. *ibid.* **36**: 368—372.

O'Kinealy, F., 1903. Localized psorosperniosis of mucous membrane of the sputum. Proc. laryng. Soc. Lond. **10**: 109.

Purandare, N. M. & S. M. Deoras, 1953. Rhinosporidiosis in Bombay. Indian J. med. Sci. **7**: 603—610.

Seeber, G. R., 1900. Un Nuevo Esporozoario Parasito del Hombre, dos Casos Encontrados en Polipos Nasales, Buenos Aires, Boullosa.

Seeber, G. R., 1912. *Rhinosporidium Kinealyi* et *Rhinosporidium Seeberi*, une question de priorité. Buenos Aires.

Trimurti, T. S., 1914. *Rhinosporidium Kinealyi.* Practitioner. **93**: 704.

Trimurti, T. S., 1924. *Rhinosporidium Seeberi* (*Kinealyi*) in a malabar woman. Lancet. **1**: 802.

Vasudevan, A., 1929. Cited by F. R. W. K. Allen & M. L. Dave. Indian med. Gaz. 1936. **71**: 376—394.

Vasudevan, A., 1932. An unusual type of parasitic infection of forearm. Indian J. med. Res. **20**: 299—302.

Wright, R. E., 1922. *Rhinosporidium Kinealyi* of the conjunctiva. Indian med. Gaz. **57**: 6—7.

Wright, R. E., 1922a. *Rhinosporidium Kinealyi* of the conjunctiva cured by tartarated antimony (tarter emetic) and notes on a case in which the lacrimal sac was affected by this sporozoon. *ibid.* **57**: 81—82.

Wright, R. E., 1922b. *Rhinosporidium Kinealyi* o fthe conjunctiva *ibid.* **57**: 82—83.

Wright, R. E. & T. S. Trimurti, 1922. *Rhinosporidium Kinealyi. ibid.* **57**: 270.

6. Blastomycosis

Andleigh, H. S., 1951. Blastomycosis in India. Indian J. med. Sci. **5**: 59.

DeMello, F. & A. Rodrigues, 1929. A case of blastomycosis. Bull. Soc. Path. exot. **22**: 142—147. Trop. Dis. Bull. **27**: 518.

Ganguli, P., 1925. Blastomycosis in the Duars. Indian. med. Gaz. **60**: 189.

Jaya Ram, S. S., M. Sirsi, V. L. Ahmad & T. K. Dayalu, 1952. Blastomycosis of lung. J. Indian med. Ass. **21**: 365.

MUKHERJEE, B. B., 1953. North American blastomycosis. J. Indian med. Ass. **23:** 22—24.
PANJA, G., 1925. A case of generalised blastomycosis. Indian med. Gaz. **60:** 475—476

7. Sporotrichosis

DEY, N. C., T. SAIKIA & C. T. MAJUMDAR. 1958. Sporotrichosis in Assam (India). Indian J. Dermat. **3:** 103—109.
GHOSH, L. M., 1932. An unusual case of sporotrichosis. Indian med. Gaz. **67:** 570.
PANJA, G., N. C. DEY & L. M. GHOSH, 1947. Fungus diseases in sporotrichosis of the skin in India. *ibid.* **82:** 200.

8. Chromoblastomycosis

ANDLEIGH, H. S., 1953. Chromoblastomycosis — a review with a favourable case. Indian J. med. Sci. **7:** 409—414.
GOKHALE, B. B., M. J. THIRUMALACHAR & A. S. PADHYE, 1959. A case of black papulosquamous skin infection of *Hormodendron* species. Symposium of fungus diseases in India.
KAKOTI, L. M. & N. C. DEY, 1957. Chromoblastomycosis in India. J. Indian med. Ass. **28:** 351—353.
RAJAN, R. V., K. C. KANDHARI & M. J. THIRUMALACHAR, 1958. Chromoblastomycosis caused by a rare yeast-like dermatiateous fungus. Mycopath. et Mycol. Appl. **9:** 5—19.
THOMAS, K., C. K. JOB & G. G. HADLEY, 1957. Chromoblastomycosis. Indian J. med. Sci. **11:** 570—73.

9. Histoplasmosis

KALRA, S. L., M. D. S. BORCAR & E. R. F. REBELLO, 1957. Histoplasmosis. Indian J. med. Sci. **11:** 496—498.
PANJA, G. & S. SEN, 1954. A unique case of histoplasmosis. J. Indian med. Assoc. **23:** 257—258.
SEN GUPTA, P. C., A. RAO, A. K. BANERJEE, A. N. CHAKRABORTY & H. N. RAY, 1957. Histoplasmosis. Bull. Cal. Sch. trop. med. **5:** 54—56.

10. Coccidiodomycoses

DAS, A. K., M. K. CHATTERJEE & B. M. DEB SARKER, 1958. A case of coccidiodomycosis in India. Indian. J. Dermat. **3;** 18—23.

ERRATUM

Vol. XIII, fasc. 1, page 72, line 38:
reads "Protomyces" for "Protomycetes".

MARY F. RIDLEY

The Queensland Institute of Medical Research
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Gallery of Contemporary Noted Mycologists

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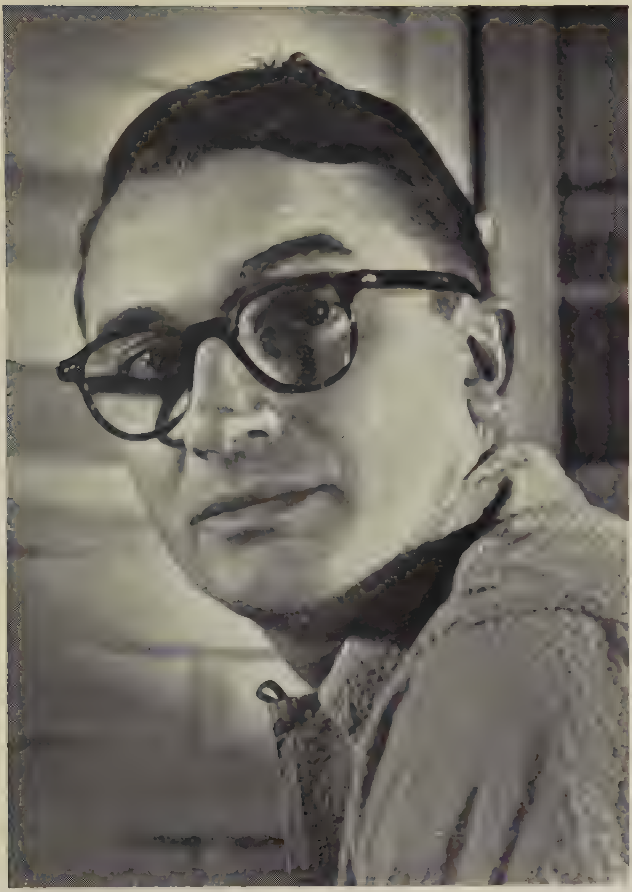

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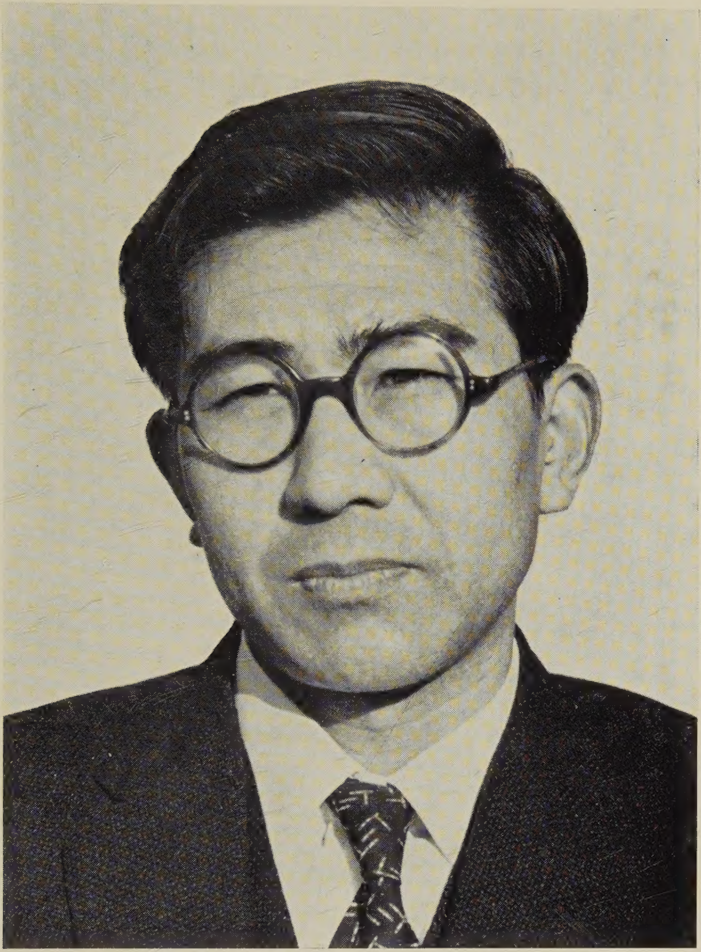

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